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VARIATIONS IN REGULATION OF ENERGY EXCHANGE ASSOCIATED WITH ESTRUS, DIESTRUS AND PSEUDOPREGNANCY IN RATS[1]

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CONSIDERATION of data which suggest that disturbances in regulation of body temperature, spontaneous locomotor activity, food intake and body weight can be produced by lesions of the hypothalamus led to proposal of the hypothesis that the hypothalamus is the level of the central nervous system most intimately concerned with integration and control of rates of energy exchange in mammals (Brobeck, 1945; Hare, 1946). The present series of experiments was designed indirectly to test the validity of this hypothesis. The experiments were based upon observations by Wang (1923) and Slonaker (1924), who found estrus in the rat to be associated with a pronounced increase in spontaneous locomotor activity, i.e., with a significant increase in energy expenditure. If the hypothesis proposed above is a valid one, the estral increase in motor output presumably arises from the hypothalamus, and one might expect to find estrus also to be accompanied by changes in food intake, body weight and body temperature, since whatever influence acts upon the hypothalamus at the time of estrus to provoke the increase in activity might be expected also to alter the other three variables. Thus, a rat might be expected to show cyclic changes in food intake, body weight and body temperature which are synchronous with the rhythm of activity previously de-

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scribed. In the present experiments an attempt to discover the existence of these other cycles or rhythms was made with a certain degree of confidence of success because Slonaker had already reported that food intake is somewhat depressed during estrus (1925), while van de Velde, and others, found the basal body temperature of women to vary with their menstrual cycle (1904; editorials, J.A.M.A., 1944, 1946). With the following procedure it was possible to confirm these observations, and to show that estrus, diestrus and pseudopregnancy are accompanied by major rearrangements in the regulation of energy exchange. During estrus the animal spends appreciably more energy than it obtains in its food, while during diestrus and pseudopregnancy it takes in more than it spends.

Procedure

Two groups of animals were used, the first of which consisted of twelve female rats of the Yale strain, maintained for 6½ months in activity cages where food intake and activity were recorded daily (rats A73–A84). Their body weight increased from a range of 145–220 grams at the beginning of the experiment to a range of 250–350 grams at its termination. During the first 4 months the room temperature was 82° F., dry bulb, while during the last 2½ months it was 64° F. The second group of animals included 11 female rats of the Sprague-Dawley strain (rats A86–A96) maintained in activity cages for 4 months, where their body weight increased from an initial range of 197–228 grams to a final range of 215–289 grams. During the first 2½ months of the experiment the room temperature was 63° F., while during the last month and a half it varied from 81 to 86° F. because of mechanical failure of the refrigeration units described below. Whereas in the first half of this period only activity and body temperature were recorded, during the last 2 months the measurements were extended to include the following: day and night activity, day and night food intake, morning (10:00 A.M.) and evening (5:30 P.M.) body weight, and morning and evening body temperature.

The various measurements were made as follows: Spontaneous locomotor activity was recorded by means of ball bearing-mounted activity cages connected to "Veeder" counters. Food and drinking water were supplied ad libitum, and food intake was estimated as the loss of weight of individual food cups containing G. L. F. "calf meal"; a special type of food cup was used to prevent spilling (fig. 15, Farris, 1942). Body weight was determined on a spring balance calibrated at 2 gram intervals. Body temperature was measured with the aid of an ordinary clinical, rectal thermometer, lubricated with mineral oil and inserted as far into the large bowel as it could be placed with gentle pressure. The thermometer usually entered approximately 3½ inches, and since its tip could be palpated just beneath the liver, it appears that the mercury bulb was in or near the upper end of the descending colon. After the rats had become accustomed to insertion of the thermometer, the lower portion of the large bowel was usually found to be empty of feces, and the thermometer usually returned as clean as it was when inserted.

In order to provide the rats with a constant ambient and radiant en-

vironmental temperature the activity cages were placed in a small "room" constructed within a larger, constant-temperature room. The larger room contained two cooling units which were thermostatically controlled; the coooling units, in turn, included two fans which by their continuous operation provided circulation of air at a constant rate. The room was unlighted except during the time measurements were being made each day.

RESULTS[2]

At intervals of 4–5 days all rats of both groups spontaneously experienced a period of hyperactivity which became conspicuous when

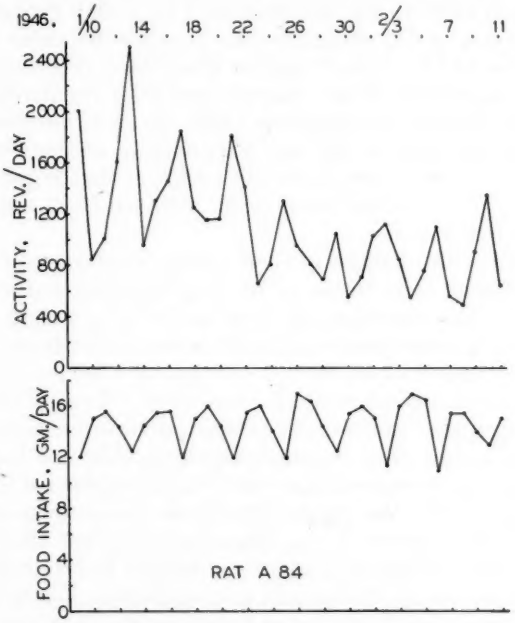

FIG. 1. Rhythms of activity and food intake. Hyperactivity during estrus is accompanied by depression of food intake.

the data were plotted graphically as in figure 1. There was a certain amount of variation from one animal to another in the length of cycles, as well as in the degree of hyperactivity (from less than 1000 rev./day in some rats to more than 10,000 in others). Nevertheless, in spite of this variation within the series as a whole, there was little variability in either the amount of hyperactivity or duration of the cycle in any

[2] Through the courtesy of Dr. James A. F. Stevenson we are able to confirm the observations of Wang (1923) and of Slonaker (1924). For a period of 3 weeks Dr. Stevenson followed the estrous cycles of eleven of our rats by means of the vaginal smear technique, and found the cycles revealed by the activity record to correspond closely with those disclosed by his examination of cells from the vagina.

given animal, and a rat which had been exhibiting peaks of 3000 rev./day each 5 days, for example, might be expected consistently to manifest this same rhythm in succeeding periods. Of the four types of rhythms studied in this experiment, the activity rhythm was the most nearly regular and predictable.

The second cycle to be studied consisted of the changes in food intake mentioned briefly by Slonaker (1925). Food intake during diestrus often exceeded intake during estrus by as much as 6 gm./day; this is the equivalent of 18 kcal./day under conditions where the total average food intake was 35–45 kcal./day, and it is evident, therefore, that the estral rhythm was accompanied by rather remarkable variations in the day to day energy intake. In a few instances the rhythm of food intake was even more regular than the activity cycle (fig. 1): in fact, the magnitude of the changes and their regularity suggested that a record of food consumption might serve as an acceptable criterion of the sex cycle of the rat. Comparison of these results with those reported by Slonaker, however, indicates that regularity of this order does not exist unless the rats are maintained at a constant environmental temperature.

Associated with changes in food intake were variations in body weight amounting to as much as 10–12 gm. during individual cycles (fig. 2). Estrus was accompanied by a loss of body weight, which was assumed to be a consequence of (i) the reduced energy intake as food and (ii) the increased expenditure as locomotor activity. This periodic loss and gain of body substance appear to be tokens of the fact that the animal is almost continually subjected to influences capable of modifying the nature of its thermodynamic equilibrium. During estrus the regulations favor expenditure over intake and the rat loses weight, while during diestrus the regulations favor intake over expenditure and a weight gain occurs. From these data it is not clear just what rôle, if any, a gain or loss of body water played in bringing about the weight loss of estrus and the weight gain of diestrus, but it seems obvious from the records of activity and food intake that the changes in body weight were primarily thermodynamic in origin, and that whatever may have been the magnitude and significance of concomitant shifts of body water, the rhythm of body weight represented primarily a gain or loss in total energy content of the rat's body.

In addition to these changes in total energy content, the estrus cycle was found to be accompanied by variations in the total amount of heat in the body. Low temperatures were consistently noted on the afternoon just before the night of hyperactivity, and were occasionally found as much as a day before or a day after estrus. As a consequence of this timing the temperature records show rhythms similar to those present in records of body weight and food intake—that is, food intake, body weight and body temperature were all lower during estrus than during diestrus. The low temperature during estrus with the

reduced body weight associated with it must have meant that the total amount of heat in the body was appreciably less than during diestrus, when both body temperature and body weight reached higher levels.

A total of 8 cases of pseudopregnancy involving 7 different rats was observed in this series, and in every case the rat ran less, ate more, and gained weight (20–25 grams in about 8 days, fig. 4). Slonaker was

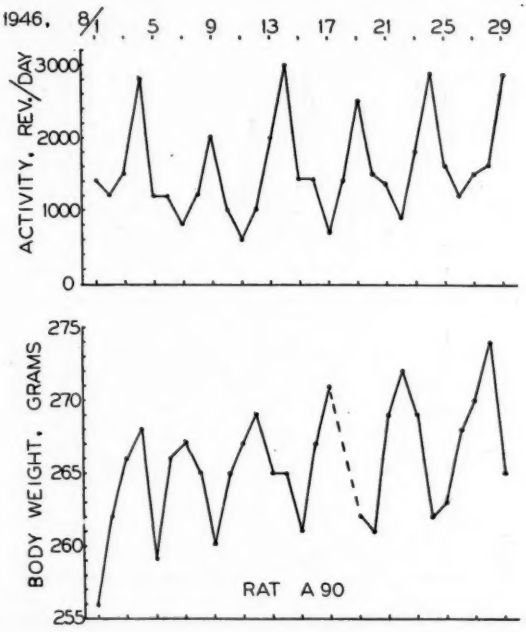

FIG. 2. Rhythms of activity and body weight. Weight gain occurs during diestrus, weight loss during estrus.

not certain that his rats always increased their food intake during pseudopregnancy (1925), but there was no doubt of it in the present series, where the rise in food intake was just as significant as the depression in spontaneous locomotor activity which he described. Moreover, gain of body weight was particularly striking because it occurred in several rats which prior to the onset of pseudopregnancy had been maintaining an almost constant weight. With termination of the pseudopregnancy reaction the food intake decreased and body weight began to fall, eventually to return to the level the rat would have been expected to attain had pseudopregnancy not intervened. (In only one instance did the rat unquestionably profit in net weight change from this reaction.) Temperature changes were less consistent than changes

in other factors, but there appeared to be a tendency toward high body temperature during pseudopregnancy, while the previously notable depressions associated with estrus disappeared for a time, until the appearance of such a depression marked the return of the normal sexual rhythm. These observations suggest that pseudopregnancy is like diestrus in that both of them represent conditions in which energy intake surpasses expenditure, with the result that a gain of

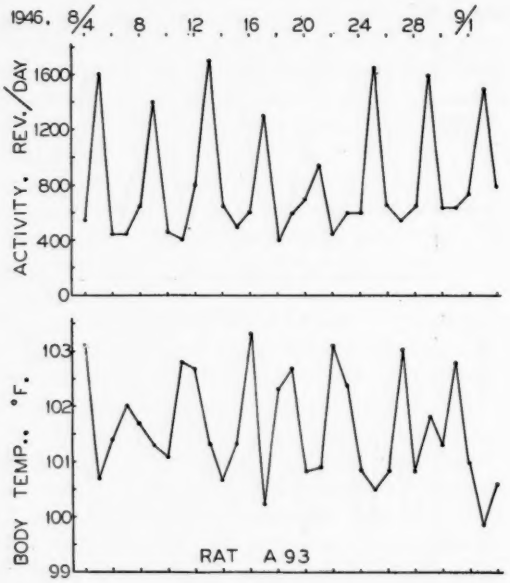

FIG. 3. Rhythms of activity and body temperature. A high temperature is associated with diestrus, a low temperature with estrus.

body weight is made possible. Both of them also appear to represent periods of net heat gain for the body. From data of this series it is impossible to say whether or not any one of these changes is primary in the sense that it gives rise to the others. No obvious, invariable sequence of events could be discovered, nor was any one cycle always associated with any other. The data seem to indicate that each of these rhythms arises independently of the others, and that all are derived from some influence which acts upon whatever cells are responsible for regulation of physiological energy exchange.

Discussion

That rhythmic variations in activity, food intake, body weight and body temperature occur coincidentally with the estral cycle of the rat is not at all surprising in view of the work of Richter (1927), Wang

(1924), Slonaker (1939), and others, all of whom have studied one or more phases of this cyclic behavior. None of these authors, however, appears to have studied all phases together, and none pointed out the significance of the observations and their relationship to the general problem of regulation of physiological energy exchange. The estrous cycle appears to be accompanied by certain basic alterations in the nature of the energy equilibrium maintained by the animal. These

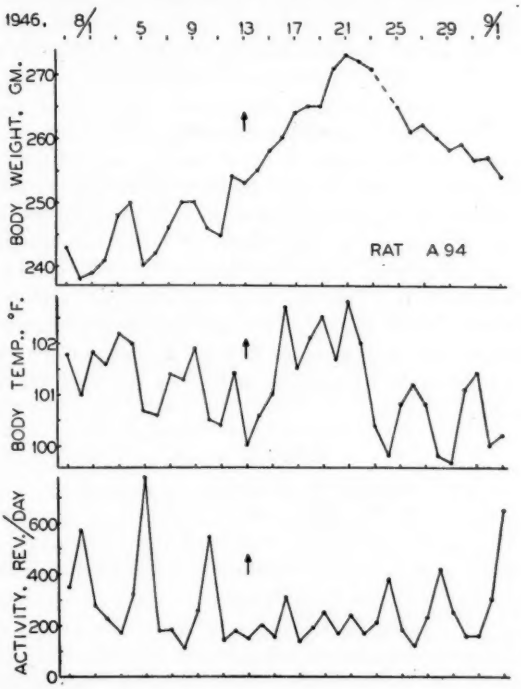

FIG. 4. Weight gain, hyperthermia and inactivity during pseudopregnancy.

alterations are probably capable of inducing changes in the intermediary metabolism (since it appears unlikely that metabolic processes will remain the same whether the output is high and intake low, as it is during estrus, or output is low and intake high, as it is during diestrus), and one might well inquire to what extent the metabolic effects generally attributed to the various sex hormones may be secondarily derived from alterations in food intake, work output or heat loss.

Although the origin of these rhythms is not clear from this study, they almost surely arise in some manner from variations in the secretory activity of either the gonads or the hypophysis—that is, from

variations in the rate of elaboration of the follicular, luteal or gonadotrophic hormones. Since the date reported here appear to be compatible with the hypothesis that the energy exchange of the body is regulated by the hypothalamus, one might consider the possibility that one or more of the hormones in question act upon the hypothalamic cells responsible for this regulation in such a way as to direct the overall energy exchange now towards the side of energy storage, now towards the side of energy expenditure. Whether this is indeed the case cannot be decided at the present time, but the problem appears to be one which lends itself to further experimental study.

Summary

Estrus, diestrus and pseudopregnancy are associated with major rearrangements in the regulation of energy exchange, since during estrus the female rat spends appreciably more energy than it obtains in its food, while during diestrus and during pseudopregnancy it takes in more than it spends. A depression of body temperature also precedes the onset of estrus. These periodic changes in the rates of energy intake and expenditure appear to be derived from either hypophysial or ovarian cycles; their presence in rats appears to be compatible with the hypothesis that processes of energy exchange are regulated and integrated by specialized cells, possibly by cells which lie within the hypothalamus.

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THE EARLY REDUCTION IN UTERINE RESPONSE TO ALPHA-ESTRADIOL IN THE PARTIALLY HEPATECTOMIZED RAT, AND THE SUBSEQUENT ENHANCEMENT DURING ACTIVE LIVER REGENERATION[1,2]

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CONSIDERABLE evidence has been marshalled in support of the hypothesis that the liver plays an important role in the inactivation of the estrogens and other steroid hormones (cf., Zondek, 1941; Burrows, 1945). Especially pertinent to the present study are the observations of a number of investigators that the response of the rat to estrogen is potentiated by toxic damage to the liver and by partial hepatectomy (e.g., Talbot, 1939; Pincus and Martin, 1940; Segaloff, 1946).

On the other hand, Szego and Roberts (1945), employing the eviscerate preparation, have demonstrated that the viscera are essential for the uterine water response to estrogen in the ovariectomized rat. This work has now been extended to the partially hepatectomized animal. The results reported below indicate that the liver is essential for estrogenic activity, and that the degree of activity is directly proportional to the rate of regeneration of hepatic tissue. The estrogenic effect of alpha-estradiol on the uterus of the ovariectomized rat was markedly reduced by partial hepatectomy carried out within 12 hours before intravenous administration of the estrogen. Thereafter, the response increased at approximately the same rate as the liver regenerated. The enhanced effect noted by other investigators was obtained when the estrogen was injected 24 to 48 hours after partial hepatectomy, during the period of most active liver regeneration.

MATERIALS AND METHODS

Experimental procedure. Female rats of an inbred Sherman strain, about 2 months old and weighing 110 to 125 gm., were ovariectomized. Approximately 2 weeks later (14 to 18 days), partially hepatectomized animals were prepared as described below. Most of the control animals were subjected to a sham operation, during which the viscera were exposed and manipulated for

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[1] Aided by grants from the Penrose Fund of the American Philosophical Society, from G. D. Searle & Co., and from the Foundation of Applied Research.

[2] Crystalline alpha-estradiol used in this study was obtained through the courtesy of Dr. E. Schwenk of the Schering Corporation.

a minute or two. Anesthetic grade ether was employed in all the operative procedures. Purina laboratory chow and water were fed *ad libitum* throughout. Body weight measurements were made at the time of hepatectomy and at the time of injection.

Alpha-estradiol in physiological saline (see below) and control saline solutions were injected into the saphenous vein in the amount of (.5 ml. per 100 gm. body weight. The time of this single injection varied in the hepatectomized group from 0 to 72 hours after partial removal of the liver. Four hours after estrogen administration, at the peak of the uterine water response to intravenously administered alpha-estradiol in the control ovariectomized rat (Szego and Roberts, unpublished results), the uteri were removed and wet and dry weight determinations were made in the usual manner(Astwood, 1938).

Measurements were obtained of the weight of liver removed at the time of hepatectomy in the partially hepatectomized group, of the amount of liver present at the time of autopsy in the same group, and of the entire liver at the time of autopsy in the control group. Estimations of the percentages of hepatic tissue removed and remaining at the time of partial hepatectomy, as well as the degree of regeneration in those animals injected 12 or more hours after operation, were made from the above measurements in a manner similar to that described by Higgins and Anderson (1931) and Brues, Drury, and Brues (1936).

Technique of partial hepatectomy. The procedure used in removing about two-thirds of the liver was similar to that employed by Higgins and Anderson (1931). Several modifications, however, were introduced.

The liver was exposed with a longitudinal mid-line incision in the ventral body wall, running caudally a distance of about 5 cm. from the center of the xiphoid process. With the aid of rubber-covered steel retractors a wide field of operation was obtained through which the liver could readily be manipulated. The median lobe was first grasped with a pair of blunt forceps and pulled outward so that most of its mesenteric attachments could be cut and a silk ligature (Deknatel, No. 4-0) slipped around its base. It was then severed and removed. The left lateral lobe was next excised in a similar fashion.

The small amount of blood which had collected in the abdominal cavity during the course of hepatectomy was carefully sponged out before the incision was closed. This blood, which amounted to approximately 1 ml., had been trapped in the liver lobes after ligation, and was the only significant source of blood loss. The abdominal musculature and peritoneum were sutured together as one layer, and the skin as another. Powdered sulfanilamide (Merck, U.S.P.) was placed between the two layers.

Mortality due to partial hepatectomy was practically zero, even when the animals were carried as long as 72 hours. Post-operatively, the rats appeared to be in very good condition, although food intake was apparently decreased for 1 or 2 days, as evidenced by the change in body weight (cf., section 4, Results). Only one animal, out of nearly 100 operated, exhibited a noticeable degree of jaundice. In this animal, the urine and most of the soft tissues including the uterus were a bright yellow.

Preparation of estradiol solutions. From a stock solution of alpha-estradiol containing 50 micrograms per ml. in 95% ethanol, the appropriate volume was pipetted into a 50 ml. glass-stoppered volumetric flask. The solvent was

evaporated to dryness in a water bath, and the residue was dissolved in 0.25 ml. of absolute ethanol. After addition of 15 ml. of 0.5 N. NaOH, followed by 1 drop of phenolphthalein (1% in 95% ethanol), the resulting solution was brought just to the acid side of the indicator with 0.5 N. HCl. Finally, this was made up to volume with distilled water, to give a solution containing alpha-estradiol, probably as the mono-sodium salt, in physiological saline at a pH close to that of blood. Appropriate dilutions were made with 0.9% NaCl. These solutions were reproducible when prepared from time to time. They were stored in the refrigerator when not in use.

RESULTS

1. *The uterine response to intravenously-administered alpha-estradiol in the adult ovariectomized rat.*

In the control series of adult rats, ovariectomized about 2 weeks previously, the uterine response to alpha-estradiol was rapid and consistent. Four hours after intravenous administration, the total uterine weight had risen from an average value of 38.7 mgm. per 100 gm. body weight to 50.2 mgm. per 100 gm. in response to 0.25 micrograms of the estrogen per 100 gm. body weight (Table I). Ten times this dose (2.5 micrograms per 100 gm. body weight) increased the uterine weight to an average value of 74.6 mgm. per 100 gm. It is apparent from the table that this rapid response was due mainly to the imbibition of water. Thus, the uterine water content rose from 79.2% in the saline injected group, to 80.7% in the group injected with the lower dose of estrogen, to 84.8% in the group injected with the higher dose. The significance of these differences in percent uterine water at successive doses was found to be very high by the Fisher "t-test" ("p" less than 0.01).

The early uptake of water by the uterus has previously been demonstrated in the immature rat in response to subcutaneously injected estrone and alpha-estradiol (Astwood, 1938), and in the adult ovariectomized rat in response to alpha-estradiol administered by subcutaneous, intravenous, and topical (uterine) routes (Szego and Roberts, 1945). In all cases, as well as in the present investigation, the change in uterine water percentage has been found to be the most reliable index of early estrogenic stimulation.

The uterine dry weight data in Table I, though not completely significant, suggest that a measurable degree of true uterine growth (laying down of new tissue) occurred within 4 hours after the intravenous administration of alpha-estradiol. The increase was about 20% and 40% respectively in response to 0.25 and 2.5 micrograms of the estrogen per 100 gm. body weight.

Sham-operated and unoperated controls showed no differences in their responses to intravenously injected alpha-estradiol. The uterine water percentage of 4 sham-operated animals was found to be $84.9 \pm 1.6\%$ (mean and standard deviation), 4 hours after the administration of 2.5 micrograms of this estrogen per 100 gm. body weight. This was

TABLE I. THE IMMEDIATE EFFECT OF PARTIAL HEPATECTOMY ON THE UTERINE RESPONSE TO ALPHA-ESTRADIOL[1]

Dose (μgm. per 100 gm. body wt.)	No. of rats		Wet weight uterus (mgm. per 100 gm.) body wt.)		Dry weight uterus (mgm. per 100 gm.) body wt.)		Uterine water content (percent)		"p" value[3]
	Control	Hepatectomized	Control	Hepatectomized	Control	Hepatectomized	Control[2]	Hepatectomized[2]	
0	7	8	38.7 ±6.2 (29.7 to 48.7)	37.3 ±6.4 (29.9 to 46.7)	8.0 ±1.1 (6.6 to 9.9)	7.6 ±1.3 (6.0 to 9.3)	79.2 ±1.1 (76.8 to 80.2)	79.7±0.4 (78.8 to 80.0)	<0.3
							p=0.01	p=0.8	
0.25	6	6	50.2 ±5.7 (41.3 to 58.4)	41.4 ±4.0 (36.8 to 48.7)	9.4 ±1.1 (8.0 to 11.4)	8.3 ±0.7 (7.4 to 9.5)	80.7 ±0.2 (80.5 to 81.1)	79.9 ±0.3 (79.6 to 80.4)	<0.01
							p<0.01	p<0.02	
2.5	9	6	74.6 ±11.8 (59.0 to 95.3)	48.3 ±6.6 (41.6 to 59.2)	11.4 ±2.4 (8.7 to 15.7)	9.2 ±1.0 (7.8 to 10.6)	84.8 ±1.2 (82.7 to 86.9)	81.1 ±1.0 (80.2 to 83.1)	<0.01

[1] Results expressed as mean ± standard deviation; range in parentheses.
[2] "p" values (calculated by the Fisher "t-test") of the differences at successive dose levels in the same group are shown.
[3] Calculated from the differences in uterine water percentage between the control and hepatectomized groups at each dose level.

not significantly different from the corresponding value in 5 unoperated controls ($84.6 \pm 0.7\%$).

Intravenous administration has been employed throughout in the present study to circumvent the possibility of reduced absorption from subcutaneous sites in the partially hepatectomized preparation. As might be expected, the response was more rapid than that occurring after subcutaneous administration. The peak in uterine water response to 2.5 micrograms of alpha-estradiol per 100 gm. body weight was found to occur at 4 hours in the adult ovariectomized rat (Szego and Roberts, unpublished observations).

2. *The uterine response to alpha-estradiol injected immediately after partial hepatectomy.*

Removal of two-thirds of the liver immediately before the intravenous administration of alpha-estradiol greatly reduced the extent of the uterine response measured 4 hours later. This is shown in Table I. The response to 0.25 micrograms of the estrogen, noted in the control group, was apparently completely obliterated by partial hepatectomy. The larger dose of estrogen (2.5 micrograms per 100 gm. body weight) produced an effect on the uterus of the partially hepatectomized rat which was quantitatively similar to that produced in the control rat by one-tenth the dose.

The high significance of the differences in uterine water percentage between the control and hepatectomized groups at 0.25 and 2.5 micrograms of alpha-estradiol, respectively, is shown in the last column in Table I. It is also apparent that partial hepatectomy had no significant effect on the uterus of ovariectomized animals injected with control saline solutions (e.g., 79.2% water in the sham group, 79.7% water in the hepatectomized group; "p" of the difference, about 0.3). Uterine dehydration, then, did not occur after the removal of hepatic tissue, and was not responsible for the reduction in response to estrogen.

These results are confirmatory of those previously found in the eviscerated, ovariectomized rat, in which *all* of the liver was removed (along with the gut and associated structures). In the latter preparation, *no* uterine water response could be elicited by large doses of alpha-estradiol and estrone, administered in divided doses and by combined routes (Szego and Roberts, 1945).

3. *The uterine response to alpha-estradiol injected at varying intervals after partial hepatectomy.*

The results obtained above were in apparent contradiction to those reported by Talbot (1939), Pincus and Martin (1940), and Segaloff (1946). Thus, Talbot (1939) found that the uteri of immature rats with livers damaged by the administration of carbon tetrachloride gradually increased in size over a period of 3 or 4 days. Pincus and

Martin (1940) observed that the estrous response to subcutaneously administered estrone was potentiated in adult ovariectomized rats whose livers had been similarly damaged. Segaloff (1946) noted a greater percentage of estrous smears in response to alpha-estradiol administration (subcutaneous) in ovariectomized rats which had been

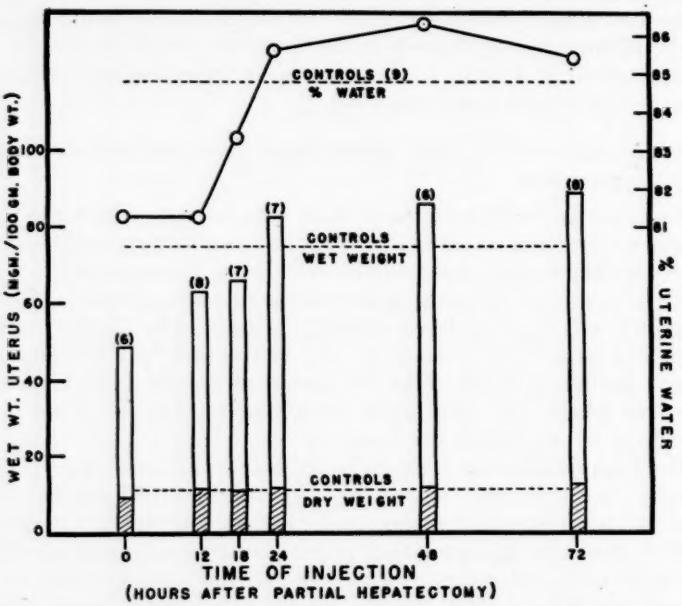

FIG. 1. The Uterine Response to Intravenous Alpha-Estradiol as a Function of Time after Partial Hepatectomy.

At the times indicated, all animals received intravenously-administered alpha-estradiol in saline, 2.5 micrograms per 100 gm. body weight. Autopsy was performed 4 hours later.

The entire height of the bar represents the average uterine wet weight at each autopsy time; the cross-hatched portion, uterine dry weight; the open portion, uterine water. All are expressed as mgm. per 100 gm. body weight. The circles on the curve represent the uterine water content in per cent. The dotted lines locate the corresponding average values observed in non-hepatectomized controls. The number of animals contributing to the average in each case is indicated by the number enclosed in parentheses.

partially hepatectomized 48 hours or more before, than in similarly-treated control animals with intact livers.

These determinations of estrogenic sensitivity, it may be noted, were not made earlier than 24 hours after toxic damage or partial removal of the liver. For this reason, it was deemed advisable to study the uterine response to injected estrogen at successive time intervals after partial hepatectomy. Accordingly, hepatectomized rats were kept warm and allowed food and water *ad libitum* for periods of time varying from 12 to 72 hours before estrogen injection. At the end of

these periods, all animals were injected intravenously with 2.5 micrograms of alpha-estradiol per 100 gm. body weight, and were autopsied 4 hours later as described above.

It was found (Figure 1) that the sensitivity of the partially hepatectomized rat to injected estrogen, though initially depressed, increased with time after operation. Estradiol, administered 0 to 12

TABLE II. THE UTERINE RESPONSE TO ALPHA-ESTRADIOL AS A FUNCTION OF TIME AFTER PARTIAL HEPATECTOMY

Group	No. of rats	Time interval[1] (hrs.)	Wet weight uterus (mgm. per 100 gm. body wt.)	Dry weight uterus (mgm. per 100 gm. body wt.)	Uterine water content	
					(percent)	"p" value[2]
Control	9	0	74.6±11.8 (59.0 to 95.3)	11.4±2.4 (8.7 to 15.7)	84.8±1.2 (82.7 to 86.9)	—
Hepatectomized	6	0	48.3±6.6 (41.6 to 59.2)	9.2±1.0 (7.8 to 10.6)	81.1±1.0 (80.2 to 83.1)	<0.01
Hepatectomized	8	12	62.8±18.1 (28.5 to 85.4)	11.6±3.2 (6.0 to 16.4)	81.1±1.3 (79.0 to 84.4)	<0.01
Hepatectomized	7	18	66.0±18.1 (45.0 to 93.3)	11.0±3.1 (7.2 to 15.2)	83.3±1.4 (80.7 to 84.8)	<0.05
Hepatectomized	7	24	82.3±16.4 (55.1 to 106.0)	11.8±2.3 (8.9 to 14.8)	85.6±1.1 (83.8 to 87.2)	0.2
Hepatectomized	6	48	86.1±15.0 (62.7 to 108.6)	11.8±2.2 (9.1 to 15.1)	86.3±0.8 (85.2 to 87.4)	<0.02
Hepatectomized	8	72	89.0±16.3 (75.4 to 117.0)	12.6±1.7 (9.1 to 14.5)	85.4±1.3 (83.5 to 87.6)	0.35

[1] Time interval between operation and intravenous injection of 2.5μ gm. α-estradiol per 100 gm. body weight. Uteri dissected out at autopsy, 4 hours after injection.
[2] Calculated from the differences in uterine water percentage between the control group and the hepatectomized groups at each time interval.

hours after liver removal, evoked a reduced uterine response, as compared with the response obtained in control rats with intact livers. Eighteen hours after partial hepatectomy, the response was similar to that obtained in the control group. At 24 to 48 hours, the uterine response surpassed that observed in non-hepatectomized animals. At 72 hours, some degree of enhanced estrogenic activity was still apparent.

The progressive increase in uterine sensitivity to exogenous estrogen in the partially hepatectomized rat,—from subnormal levels the first 12 hours after operation to supranormal levels after 1 to 2 days,—was mainly manifested by a gradually increased imbibition of water. This is illustrated in Table II, wherein it may be seen that the average uterine water content increased from a low level of 81.1% in animals injected immediately or 12 hours after liver removal, to an intermediate level of 83.3% 18 hours after operation, to 86.3% at 48 hours. The limits of significance between these values and the control value of 84.8% in control rats injected with the same dose of estrogen are

shown in the last column of the table. It is evident that both the depression at 0 and 12 hours, as well as the enhancement at 48 hours at least, are highly significant.

4. *The rate of liver regeneration in the partially hepatectomized rat.*

The rate of restoration of the hepatic tissue remaining after partial hepatectomy is shown in Table III. It may be seen that the most rapid increase in hepatic tissue occurred toward the end of the first day. Thus, in the 6-hour period between 22 and 28 hours after operation,

TABLE III. THE RATE OF LIVER REGENERATION AFTER PARTIAL HEPATECTOMY

Group	No. of rats	Autopsy[1] time (hrs.)	Liver present		Body weight		
			gm./100 gm. body wt.	% normal liver wt.	operation (gm.)	autopsy (gm.)	% loss[1]
Control	32	—	4.46 ±0.49	100.0	—	153 ±13	0
Hepatectomized	69	0 to 4	1.53 ±0.40	35.0	—	149 ±15	0
Hepatectomized	8	16	1.99 ±0.12	44.6	148 ±17	137 ±20	7.4
Hepatectomized	7	22	2.14 ±0.13	48.0	146 ±13	132 ±14	9.6
Hepatectomized	7	28	2.81 ±0.26	63.3	161 ±2	143 ±3	11.2
Hepatectomized	6	52	3.24 ±0.22	73.0	160 ±18	145 ±16	9.4
Hepatectomized	8	76	3.51 ±0.23	79.1	148 ±7	142 ±7	4.1

[1] From time of operation to time of autopsy.

the amount of liver present increased from 48.0% of the normal weight to 63.3%. At all other time intervals, the rate of regeneration was much less rapid. This phenomenon is also illustrated in Figure 2 (open circles). These results are similar to those of Higgins and Anderson (1931) and Brues, Drury, and Brues (1936). The latter workers further found that the mitotic rate was highest during the second day. The early and most rapid increase in liver size is apparently due to an enlargement of the hepatic cells; the more gradual later increase seems to be due to cell division (cf., Norris, Blanchard, and Povolny, 1942).

The change in the gross appearance of the liver tissue remaining after partial hepatectomy was striking. During the period of lag in liver regeneration which ensued for 16 to 22 hours after operation (Table III), the liver assumed a pale yellow appearance, which became almost white by 28 hours. It is probable that the paling was due to the accumulation of fat, since the water content (Higgins and Anderson, 1931) and the nitrogen content (Brues, Drury, and Brues, 1936) of the liver both decrease the first day after liver removal. In the present study, the gross appearance of the liver was essentially normal after 2 to 3 days.

It may be noted that a transient gradual decrease in body weight occurred after partial hepatectomy. This loss reached a maximum of

11.2% after 2 days, and was then followed by a rapid recovery (Table III). Somewhat comparable data have been presented by Higgins and Anderson (1931).

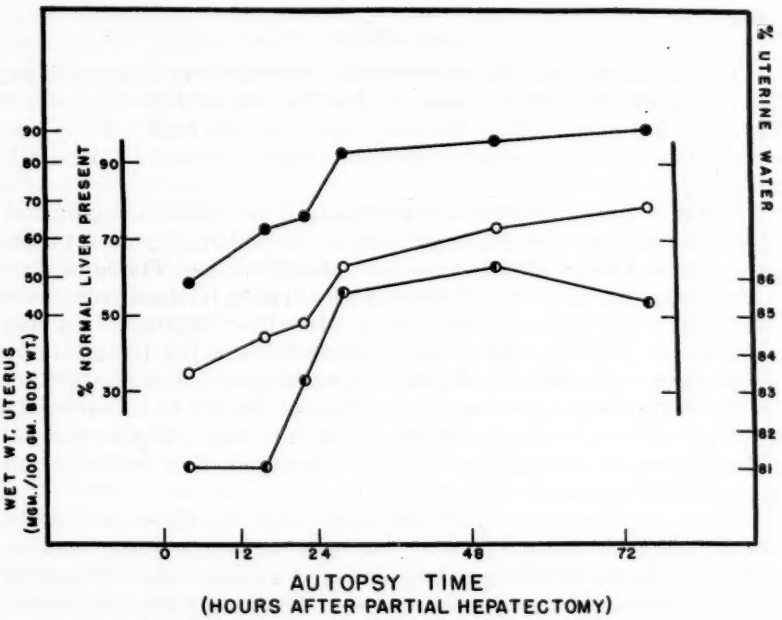

FIG. 2. The Temporal Correlation between Liver Regeneration and Uterine Response to Intravenous Alpha-Estradiol after Partial Hepatectomy.

The intravenous injection of alpha-estradiol in saline, 2.5 micrograms per 100 gm. body weight, in all cases preceded the autopsy time by exactly 4 hours.

Open circles represent the amount of liver present expressed as per cent of normal. Solid circles represent uterine wet weight in mgm. per 100 gm. body weight. Half-filled circles represent the percentage uterine water content. Each point is the average of at least 6 to 8 animals.

5. *The positive correlation between liver regeneration rate and the uterine response to alpha-estradiol in the partially hepatectomized rat.*

A close parallelism was found to exist between the temporal increase in liver weight which occurred after partial hepatectomy and the increase in uterine sensitivity to intravenously-injected alpha-estradiol in the same animals. This is shown in Figure 2.

It is apparent that the uterine weight response (solid circles, Figure 2) to 2.5 micrograms of alpha-estradiol per 100 gm. body weight increased at the same rate after partial hepatectomy as the weight of the hepatic tissue (open circles). In each case, there was an initial lag for nearly 1 day after removal of two-thirds of the liver. This was succeeded by a very rapid rise for a few hours, after which the rate of increase became more gradual.

The increase in the percent uterine water response to estrogen with time after partial hepatectomy progressed in a manner essentially similar to that of the uterine weight response. In this case, however, the parallelism with the rate of liver regeneration was not quite as striking.

DISCUSSION

It is apparent from the above results that the liver is essential for the estrogenic effect of alpha-estradiol on the uterus of the adult ovariectomized rat. For 12 to 18 hours after partial hepatectomy the response to the intravenously injected estrogen was found to be much reduced.

In the otherwise normal, ovariectomized rat, 0.25 micrograms of alpha-estradiol per 100 gm. body weight resulted in a significant uterine response 4 hours after intravenous administration. The same dose had no effect on the uterus of the partially hepatectomized, ovariectomized rat when injected immediately after liver removal. In a like manner, ten times the above dose (2.5 micrograms per 100 gm. body weight) caused in the control group a large degree of water imhibition by the uterus and a significant laying down of new tissue. Immediately after partial hepatectomy, the latter dose had only a slight effect on the uterine water content and a similarly reduced effect on the actual growth of the uterus.

These observations confirmed and extended those previously noted in the eviscerate. The latter was found to be completely insensitive to large doses of alpha-estradiol and estrone administered in divided doses and by combined routes. No evidence for uterine water imbibition could be obtained in this preparation over periods of time as long as 24 hours after estrogen administration (Szego and Roberts 1945).

Seemingly opposed to the above is the large amount of data indicating that estrogenic sensitivity was enhanced by damage or removal of the liver. As has been noted previously, these observations were made at periods of time not earlier than 24 hours after liver damage (Talbot, 1939; Pincus and Martin, 1940; Segaloff, 1946). In the present investigation, when the uterine response to alpha-estradiol was studied at varying periods of time after partial hepatectomy, it was found that the sensitivity to estrogen was enhanced above the normal after 1 to 2 days. Thus, the uterine water response rose from subnormal levels soon after hepatectomy, to values not significantly different from the normal in 18 to 24 hours, to definitely enhanced levels in 48 hours. The dry weight response followed a similar progression, but at a less significant level.

It would appear from these results that the liver has a dual role in estrogen metabolism, functioning both in the "activation" and inactivation of these steroid hormones. If this is true, it must be assumed that both processes are almost completely suppressed at the time of partial hepatectomy, and that the "activation" function returns

more rapidly than the inactivation process as the liver regenerates.

The results obtained in the present study, however, can also be explained without invoking the theory of estrogen inactivation by the liver. The fact that the increase in estrogenic activity with time after partial hepatectomy parallels the rate of regeneration of the liver suggests that a single process may be responsible both for the early reduction and subsequent enhancement in uterine response. A progressive increase in the enzymic or other metabolic activities involved in the "activation" of alpha-estradiol, gradually climbing from a subnormal level soon after partial removal of the liver to a supranormal peak 48 hours later, could result in the phenomena observed.

The theory of estrogen inactivation by the liver is generally accepted, and a large body of literature has grown up in support of it. It is not possible here to discuss all of this work in the light of the present observations. It may be mentioned, however, that the disappearance of estrogen from blood or other media either perfused through the liver (Israel, Meranze, and Johnston, 1937), or injected into the body (Zondek, 1934), or incubated with liver slices or extracts (e.g., Heller, 1940) need not be due to true inactivation, but merely to a change in the chemical state of the estrogen (see below). In addition, a significant proportion of the estrogen apparently inactivated by the liver *in vivo* may undergo an enterohepatic circulation as suggested by Cantarow et al. (1942, 1943).

It is possible that the "activation" of exogenous and endogenous estrogen is accelerated in the livers of animals subjected to the stresses of inanition and carbon tetrachloride as well as partial hepatectomy. In all cases, it seems likely that the hepatic tissue present may be undergoing some sort of regenerative process. Accelerated "activation," then, rather than depressed inactivation of estrogen, may be offered as an alternative explanation for the enhancement in estrogenic activity obtained by other workers in partially hepatectomized rats (Segaloff, 1946), in rats with livers damaged by carbon tetrachloride (Talbot, 1939; Pincus and Martin, 1940), and in rats with livers damaged by various types of inanition (Biskind and Biskind, 1942; Drill and Pfeiffer, 1946; etc.)

A number of investigators have demonstrated that estrogenic material is less effective when implanted in the portal field than when implanted in other areas (Golden and Sevringhaus, 1938; Biskind and Mark, 1939; Segaloff, 1943). This would seem to offer strong evidence in support of the inactivation hypothesis. The possibility should not be overlooked, however, that the spleen *in situ* or mesenteric tissue *per se* may be able to inactivate or otherwise prevent the estrogen from exerting its effects on vaginal estrus (cf., Zondek, 1941).

Whether or not estrogen activation *per se* takes place in the liver remains to be demonstrated. It is equally possible that the decreased effect of estrogen on the uterus of the ovariectomized rat, noted soon after partial or complete removal of the liver, might be due to the

lack of some metabolite normally supplied by the liver and essential for the response (cf., Szego and Roberts, 1945). If such were the case, the response of the liver might be due either to direct stimulation of the liver by the estrogen, or to an indirect demand on the liver for its products by the estrogen-stimulated uterus. In support of the hypothesis that the liver supplies some metabolite essential for estrogenic activity is the work of Hertz (1945). His results point to the presence of active forms of folic acid and possibly other substances in liver extracts fed by mouth, which appeared to be essential for the complete oviduct growth response to injected estrogen in the chick.

Interconversion of the free estrogenic steroids by the liver may be involved in the "activation" of alpha-estradiol. Although Heller (1940) found that alpha-estradiol was destroyed without further interconversion by liver mince, Schiller and Pincus have concluded from observations on the perfused liver (1943) and the partially hepatectomized male rat (1944) that alpha-estradiol can be converted to estrone and estriol by the rat liver. There is no evidence, however, that such a conversion is necessary for estrogenic activity. As a matter of fact, estrone has been found to be completely ineffective in the eviscerated rat (Szego and Roberts, 1945), and estriol exhibits a reduced effectiveness similar to that shown by alpha-estradiol in the partially hepatectomized, ovariectomized rat (Szego and Roberts, unpublished observations). Estriol or estrone formation by the liver then, although it may be involved, cannot be the only factor in the "activation" of alpha-estradiol.

On the other hand, suggestive evidence has been reported for the possible presence of an estrogen-activating mechanism in the liver (Szego and Roberts, 1946; Roberts and Szego, 1946). It has been found that the form of the estrogen present in the blood of a number of species, including man, differs from the free steroid. In general, natural circulating estrogen appears to exist as an estrogen-protein complex (estroprotein), in which the estrogen is tenaciously bound to the specific protein in an hydrophilic esterified form. In the human, it seems likely that this combination consists of estriol, probably esterified as the glucuronide and linked to a beta-globulin found in the plasma beta-lipoprotein fraction (cf., Roberts and Szego, 1946). It is possible that the liver functions in the formation of this complex, which may be necessary for the transport and activity of endogenous and exogenous estrogen.

SUMMARY

The uterine response to intravenously administered alpha-estradiol was greatly depressed by partial hepatectomy carried out 12 to 18 hours previously. This was evidenced particularly by a decreased imhibition of water, but also by a decreased growth by the

uterus of the partially hepatectomized, ovariectomized rat in response to doses of this estrogen of 0.25 and 2.5 micrograms per 100 gm. body weight. The reduction in sensitivity to alpha-estradiol immediately after liver removal was of the order of 90%.

When the uterine response was studied at intervals after partial hepatectomy, it was found that the sensitivity to estrogen gradually increased. Thus, 18 to 24 hours after operation the response had returned to normal. In 48 to 72 hours, an enhanced effect was noted.

The increase in uterine sensitivity to estrogen with time after partial hepatectomy was observed to parallel the rate of liver regeneration in a striking fashion. For example, the early lag in the restoration of hepatic tissue for the first 22 hours after operation corresponded to a similar lag in the uterine response. In both cases, a rapid increase was noted between 22 to 28 hours. Thereafter, the increase was more gradual in both uterine response and liver restoration.

The results obtained indicated that the liver was essential for the estrogenic effect of alpha-estradiol on the uterus of the ovariectomized rat. They also suggested that accelerated estrogen "activation" rather than inactivation was responsible for the later enhanced effect observed after partial removal of the liver.

The theories of estrogen "activation" and inactivation have been discussed.

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THE RATE OF TURNOVER OF PROTEIN-BOUND IODINE IN THE PLASMA OF THE DOG AS MEASURED WITH RADIOACTIVE IODINE[1]

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THE METHODS most frequently employed for estimating the rate of thyroid secretion have been based on the replacement of circulating thyroid hormone by the injection of crystalline thyroxine. In the procedure introduced by Dempsey and Astwood (1943), rats were fed thiouracil in order to inhibit the formation of the thyroid hormone and injected simultaneously with graded doses of l-thyroxine. The minimum quantity of thyroxine necessary to prevent increased thyrotropic activity of the pituitary as judged by thyroid size was taken as equivalent to the rate of secretion of the thyroid hormone. This amounted to 5.2 micrograms of l-thyroxine per day for 80–120 gm. rats kept at 25° C.

The above method was applied to the chick by Mixner *et al.* (1944), and to the fowl, goat, and cow by Schultze and Turner (1945).

Griesbach and Purves (1945) employed subtotally thyroidectomized young rats to estimate the normal rate of thyroxine secretion. When less than 2.25 micrograms of dl-thyroxine was injected daily into such rats, hypertrophy of the remaining thyroid fragment occurred. When more was administered, atrophy of the thyroid fragment ensued. A daily supply of 2.25 micrograms dl-thyroxine per 100 gm. body weight was therefore taken to be equivalent to the normal daily secretion of thyroid hormone. Interestingly enough, these workers found that the same quantity of thyroxine was required to inhibit basophilic changes in the pituitary glands of normal rats treated with thiourea.

Other methods for determining the rate of thyroid secretion have been reviewed by Schultze and Turner (1945). Fishburne and Cunningham (1938) reported that it required daily injections of about 40 micrograms of crystalline thyroxine to restore to normal the heart rate of thyroidectomized rats. Rowlands (1942) stated that only 2.5 micrograms of crystalline thyroxine were required to restore normal growth in 50 gm. thyroidectomized rats. From the iodine loss in the gland of the thiouracil-treated rat, Astwood and Bissell (1944) concluded that this animal secretes hormonal iodine at a rate of approximately 3 micrograms per day (equivalent to 4.8 micrograms of thy-

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roxine), a value that is in close agreement with that reported by Dempsey and Astwood (1943).

A new approach to this problem is offered in the present communication. It has been demonstrated that in the rat injected radioactive iodine is rapidly incorporated into the protein-bound iodine fraction of plasma, and evidence was presented for the view that this *protein-bound radioactive iodine* is closely identified with circulating thyroid hormone. (Chaikoff, Taurog and Reinhardt, 1947.) This finding opened the possibility of applying the method of Zilversmit *et al.* (1943) to the measurement of the turnover rate of the circulating thyroid hormone. The latter is obtained in a labeled form by injecting donor animals with radioactive inorganic iodine and separating plasma from these animals at a suitable interval thereafter. This plasma containing the radioactive protein-bound iodine is then injected into recipient animals and its rate of disappearance from their circulation determined.

EXPERIMENTAL

The success of the present experiment depends upon the ability of the donor animal to convert injected radioactive inorganic iodine into protein-bound iodine of plasma. It is necessary that the activity of protein-bound iodine in the plasma of the donor animal be high enough so that its measurement is still possible after its dilution when injected into the recipient animal. It was not found possible to attain the required activity when dogs were used as donor animals. However, since the rat is able to tolerate a relatively much larger dose of I^{131} than the dog, it was found possible with these animals to obtain plasma containing the required activity in the protein-bound iodine fraction. As indicated in a previous report from this laboratory, the rat on a low iodine-intake converts injected inorganic I^{131} rapidly into protein-bound iodine, and 24 hours after the injection approximately 90 per cent of the plasma's radioactivity is in protein-bound form. (Chaikoff, Taurog and Reinhardt, 1947.)

Rats of the Long-Evans strain weighing 200–300 gm. were used as donors. They had been raised since weaning on a diet that contained 0.3 micrograms of iodine per gram. Each rat was injected with 50–75 microcurries of I^{131} (as inorganic iodide). They were sacrificed 24–30 hours after the injection. Blood was taken directly from the heart, heparin being used as anticoagulant. Plasma from 10–12 rats was pooled to provide enough material for injection into each recipient.

Since it was necessary to remove 5 or 6 samples of blood from each recipient animal,[2] dogs were used as recipients rather than rats. The

[2] In preliminary experiments, an attempt was made to use rats as recipicents, 3 or 4 rats being used at each time interval during the 6-hour period of observation. Since a large fraction of its blood had to be removed for a single determination, each rat was used only once. The spread of values at each time interval was so great, however, that the use of the rat as recipient was abandoned.

dogs suffered no observable ill effects after being injected intravenously with 40–50 cc. of rat plasma,—not even hemolysis of red blood cells.

Blood samples were taken from the dogs at approximately hourly intervals after the injection. The plasma was separated and duplicate samples taken for the determination of their radioactivity. Total radioactivity was measured by placing the plasma in copper boats, adding 2 drops of 2N NaOH to prevent loss of volatile iodine, drying the sample on a hot plate, and counting the dry sample with an aluminum wall counter.

The activity of the donor plasma was sufficiently high so that no mass-absorption correction had to be applied to the small quantity of this plasma used for determination of its radioactivity. The radioactivity removed from the recipient dogs, however, was so dilute that it was necessary to use as much as 2 cc. in order to measure it. In this case the mass-absorption was quite appreciable, and a correction was applied to each measurement.

THEORY OF METHOD

Turnover rate of protein-bound iodine is defined as the rate at which newly formed protein-bound iodine appears in plasma. Since the composition of plasma remains constant, this rate of appearance is equal to the rate of disappearance of plasma protein-bound iodine.

Turnover time is the time required for the delivery or disappearance of a amount of protein-bound iodine equal to that present in the whole circulating volume.

The relation between the rate of decrease in the concentration of a radioactive compound in plasma and its turnover time (t_t) has been formulated by Zilversmit *et al.* (1943) who showed that

$$t_t = -\frac{\log e}{s},$$

where s is the slope of the straight line obtained by plotting the logarithm of the radioactivity per unit volume against time.

By noting that this slope is equal to $-\frac{\log 2}{t_\frac{1}{2}}$, this expression may be transformed into the following more convenient equation:

$$t_t = 1.44\ t_\frac{1}{2}.$$

$t_\frac{1}{2}$ is the time required for the original activity to reach half its initial value, and this time can be readily obtained by inspection of the curves.

The turnover rate of protein-bound iodine in plasma is related to its turnover time as follows:

$$\text{turnover rate} = \frac{\text{total protein-bound iodine in circulating volume}}{\text{turnover time}}.$$ In order to use this relation it is necessary to know the circulating volume of the recipient

dog. The latter can be obtained from the logarithmic plot by extrapolating the line back to zero time. The intercept represents the radioactivity per unit volume at zero time and the circulating volume is obtained by dividing the total counts injected by the intercept value.

In the above calculations no account is taken of the inorganic I^{131} injected along with the protein-bound I^{131}. Since the inorganic I^{131} amounted to 5–14 per cent of the total, some error was introduced by neglecting this fraction. It has been assumed in the calculation of *turnover rate* that plasma protein-bound iodine consists primarily of a single iodine compound attached to protein.

RESULTS

The results obtained with 5 normal dogs are shown in fig. 1. All dogs except Dog A had been fasted for 24 hours. Dog A was fed 5 hours before the injection of the radioactive plasma.

TABLE 1. SUMMARY TABLE OF DATA OBTAINED FROM RECIPIENT DOGS

Dog		A	B	C	D	E
Weight of dog (kg.)		8.5	8.0	9.5	8.1	9.2
Volume of plasma injected (cc.)		41	42	44	40	50
Radioactive units injected	Total (counts per minute)	6.84×10^5	4.62×10^5	2.34×10^5	2.81×10^5	4.98×10^5
	Protein bound (counts per minute)	6.37×10^5	3.96×10^5	2.03×10^5	2.44×10^5	4.75×10^5
Radioactive units per cc. of plasma at zero time (counts per minute)		1180	615	345	400	615
Circulating volume (cc.)		540	644	590	610	772
Protein-bound iodine level of plasma (micrograms per cent)		3.2	2.6	2.2	3.3	3.1
Turnover time (hours)		6.2	4.0	6.5	7.0	7.5
Turnover rate (micrograms of iodine per 24 hours)		67	100	48	69	77
Turnover rate per kg. body weight per 24 hours		7.9	12.5	5.1	8.5	8.4

The rate of decrease of plasma radioactivity is seen to approximate a logarithmic relationship. The turnover times and turnover rates calculated from these curves as described above are shown in Table 1. The *turnover times* vary from 4 to 7.5 hours and the *turnover rates* from 50 to 100 micrograms per 24 hours. Expressed on a per kg. basis the turnover rates vary from 5.1 to 12.5 micrograms per 24 hours.

The circulating volumes calculated from the curves in fig. 1 are also shown in Table 1. These volumes turn out to be somewhat higher than the expected plasma volume. Since the dogs were injected with 40–50 cc. of plasma, this may explain, in part at least, the abnormally high circulating volume. It is possible also that injected radioactive protein-bound iodine distributes itself through a volume slightly larger than the plasma volume as determined by dye methods.

DISCUSSION

In a previous communication from this laboratory (Chaikoff, Taurog, and Reinhardt, 1947) it was shown that very little protein-bound iodine is formed in the absence of the thyroid gland. Because of this finding the assumption has been made here that the turnover

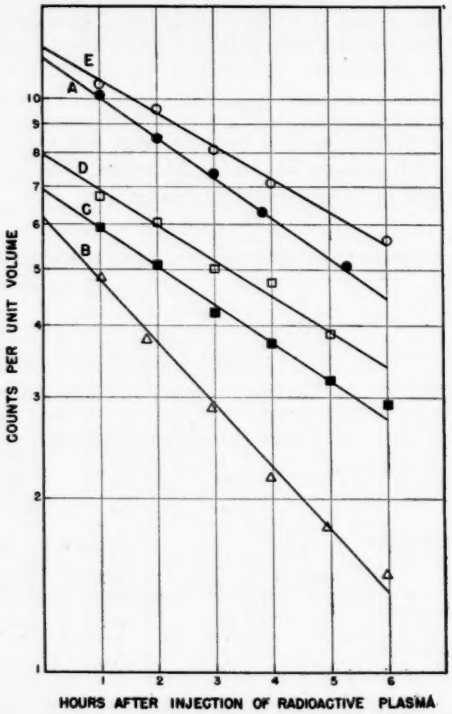

FIG. 1. The disappearance of injected radioactive protein-bound iodine from plasma of the dog. The ordinate scale is logarithmic.

rate of protein-bound iodine of plasma is very nearly equal to the rate of secretion of hormonal iodine by the thyroid.

It is of interest to compare the values obtained here for the turnover rate of protein-bound iodine with those reported for the rate of secretion of thyroid hormone. As pointed out above the latter has been measured by replacement of the circulating hormone with injected crystalline thyroxine. Unfortunately, no such measurements have been made for the dog. From the point of view of body weight, however, the dogs of the present investigation may be compared with the young goats studied by Schultz and Turner (1945). In these goats (average weight 21.8 lbs.) the thyroxine secretion rate was equated to an injection rate of 180 micrograms of dl-thyroxine per day. Since

the d-form of thyroxine is believed to be inactive (Reineke and Turner, 1945), this is equivalent to 90 micrograms of l-thyroxine or to 59 micrograms of thyroxine iodine per day. This value compares favorably with the average figure obtained by the present method, namely 72 micrograms of protein-bound iodine per day for dogs averaging 19 lbs.

In a study in which radioactive iodine was used to determine the specific activity-time relationships of thyroxine and diiodotyrosine in the thyroid gland of the dog, Mann *et al.* concluded that 1.5 per cent of the thyroxine in the gland is renewed every hour. Interestingly enough, this would yield turnover rates varying from 24 to 240 micrograms of thyroxine iodine per day for their dogs. The significance of these findings will be dealt with in a subsequent communication.

SUMMARY

The application of the radioactive isotope of iodine I^{131} to the measurement of the turnover rate of protein-bound iodine of plasma is described.

In 5 dogs weighing 8–10 kilos, the turnover rates were found to be 50–100 micrograms per 24 hours. The formation of an amount of protein-bound iodine of plasma equal to that contained in the whole circulating volume (turnover time) occurred every 4 to 7.5 hours.

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THE EFFECT OF HYPERGLYCEMIA ON INSULIN SECRETION AS DETERMINED WITH THE ISOLATED RAT PANCREAS IN A PERFUSION APPARATUS[1]

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VARIOUS techniques have been devised in an effort to study the factors which directly influence insulin secretion. Zunz and La Barre (1927) joined two dogs by a pancreatic-jugular anastomosis and observed the effect of pancreatic blood of the donor animal upon the blood sugar of the recipient animal. Gayet and Guillaumie (1933) and Foglia and Fernandez (1935) transplanted the pancreas from one dog into the neck of another dog which had been previously depancreatized and observed the blood sugar changes in the latter animal. London and Kotschneff (1937) placed an angiostomy cannula in the pancreato-duodenal vein and determined the glucose and insulin content of blood from the pancreas after a glucose meal. By these various methods each group of workers has shown, in turn, that hyperglycemia stimulates the secretion of insulin directly. Zunz and La Barre found the denervated pancreas less sensitive, however, to the stimulating effect of hyperglycemia.

In order to study some of the hormonal factors which influence insulin secretion a technique has been developed by which the pancreas of the rat can be removed from the body and maintained functionally active in a small perfusion apparatus. This paper deals with a description of the technique and furnishes additional proof that hyperglycemia stimulates the secretion of insulin directly. The direct effect of hormones on insulin secretion will be reported in a second paper (Anderson and Long, 1947).

METHODS

Perfusion Apparatus. A pulsating perfusion apparatus, which had been designed and built by one of the authors (Long), was used for this study. A photograph of the apparatus is shown in Fig. 1; the detailed description have been published previously (Long, 1946).

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Insulin Assay. The method used for detecting insulin in the blood which had been circulated through the pancreas was the assay method described by Anderson, Lindner and Sutton (1947).

Operative Technique. The animals used in these experiments were normal male rats which had been fasted for approximately 17 hours, at which time the insulin content of the pancreas was presumed to be at a minimal level (Haist, 1944). Nembutal anaesthesia was employed. The stomach and

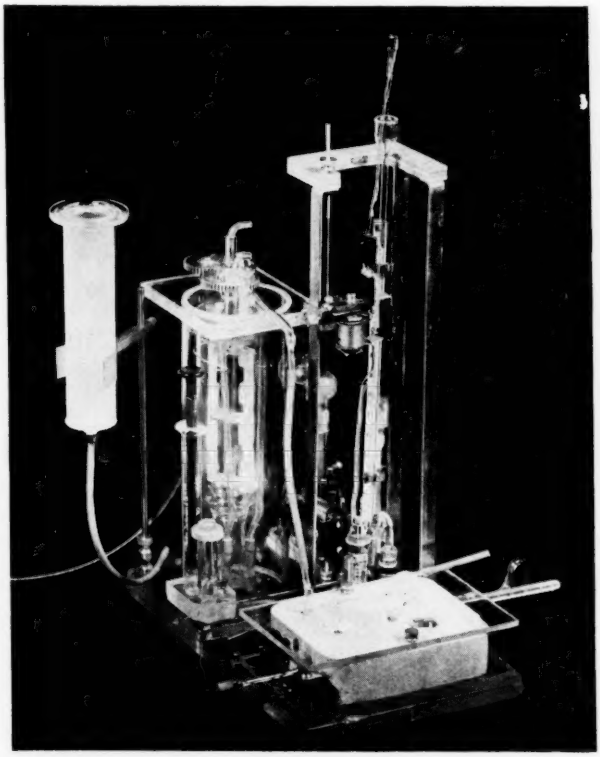

FIG. 1. Photograph of the perfusion apparatus. The chamber in which the pancreas, stomach and duodenum are placed, is in the foreground.

duodenum and their mesentery, which contains the pancreas scattered through it, were dissected free from the surrounding tissues. During the operation great care was taken to keep the animal's body warm, to cover the viscera with moist, warm cotton plaques, to avoid traumatizing the tissues by the instruments, and to securely ligate or cauterize all the vessels severed in the operation.

Cannulae were placed in the esophagus and the distal end of the duodenum so that these organs, when placed in the chamber, could be connected with tubes leading through the wall of the chamber to the outside.

A cannula was then inserted into the portal vein just distal to the veins draining the pancreas and immediately 0.25 cc. heparin was injected through this cannula. The aorta then was freed from the posterior body wall, and all its branches were ligated except for the coeliac and superior mesenteric arteries, whereupon a cannula was placed in the aorta just below the superior mesen-

TABLE 1. PROTOCOL OF AN EXPERIMENT

Date Jan. 25, 1946
Experiment #34
Rat W6027 normal male fasted 17 hours
Weight 180 gm.
Apparatus *circulating blood:* 12 cc.
plasma in chamber: 6 cc.

Time	Operative Procedure			
8:20 A.M.	nembutal 0.18 cc. (6% solution)			
8:30	operation started			
9:57	portal vein tied and cut			
9:58	aorta tied and cut			
10:00	organs in chamber, pump going			
	Blood pressure (mm. Hg.)	Temperature	Rate of flow (cc./min.)	Blood sugar in perfusate (mm. %)
10:09	110	101° F.	2.25	72
10:20	2 cc. blood added to circulation			
10:33	110	100.6° F.	1.5	44
	3 cc. blood removed for insulin assay I*			
	2 cc. blood and 40 mg. glucose added			
10:47			2.7	240
10:55	40 mg. glucose added to blood			
11:05	110	100.5° F.	2.7	360
11:37	3 cc. blood removed for insulin assay II			
	4 cc. blood and 40 mg. glucose added			
11:40				364
11:55	110	100.6° F.	1.5	320
12:00	40 mg. glucose added			
12:37	3 cc. blood removed for insulin assay III			
	Experiment stopped			

* Insulin assay I + 39 mg. %
Insulin assay II − 57 mg. %
Insulin assay III −103 mg. %

teric artery. The final step in the operation was to tie and cut the portal vein adjacent to the liver and tie and cut the aorta at a level just above the stomach. Two or three minutes usually elapsed between the final ligation and cutting of the vessels and the reestablishment of the circulation after placing the organs in the perfusion apparatus.

Procedure of the Experiment. The blood used for the perfusion was freshly drawn from rats which had been injected with heparin. Plasma was used in the specimen chamber to bathe the organs. Both the blood and plasma were from rats which had been fasted for about 17 hours, and did not contain any detectable insulin. During the course of an experiment the temperature in the specimen chamber and the pressure of the perfusion pump were kept at a fairly constant level. The rate of blood flow was checked every 10 minutes. The appearance of the tissues and the circulation in the

blood vessels were watched under a microscope during the experiment. Oxygenation of the blood was accomplished by spreading the blood in a thin film on the wall of a cylindrical reservoir and blowing oxygen over it. A rubber strip set in a frame like a wind-shield wiper was used to spread the blood.

A copy of a protocol (Table 1) serves as an outline of the procedure followed during the course of an experiment. It will be noted that the level of glucose in the perfusate influenced the rate of blood flow through the organs. During the first 35 minutes, when the blood sugar dropped from 72 mg. % to 44 mg. %, the rate of blood flow decreased from 2.25 cc. per minute to 1.5 cc. per minute, although the pump maintained a constant pressure of 110 mm. Hg. and the temperature in the chamber was almost constant around 101° F. When glucose was added so that the blood glucose level was raised to 240 mg. %, the rate of flow increased to 2.7 cc. per minute, which was the maximum rate of flow in these experiments.

The rate of disappearance of glucose seemed to increase as the level of glucose was raised in the perfusate. In order to maintain a blood glucose of 350 mg. %, 80 mg. of glucose were added per hour to the circulating blood. Circulation would frequently begin to fail after 3 to 4 hours of perfusion. Hemolysis of the blood was believed to be one of the causes of this failure.

EXPERIMENTAL DATA

The response of the islet cells to an elevated blood sugar is shown in the protocol (Table 1). During the first 35 minutes of the perfusion, the blood sugar level was allowed to drop from the fasting level of 72 mg. % to a hypoglycemic level of 44 mg. %. A test was made for insulin at the end of this period. This was done by injecting 3 cc. of the blood perfusate intravenously into an adrenodemedullated diabetic hypophysectomized (ADH) rat according to the insulin assay technique previously referred to. The change in blood sugar of the test animal (ADH rat) was +39 mg. %, which is interpreted as *no insulin* present in the perfusate. Glucose was then added to the circulating medium and the perfusion of the pancreas continued. The blood sugar level was increased to 240 mg. % and finally to 360 mg. % by the addition of 80 mg. of glucose. The insulin assay test after perfusion for one hour showed a change in the blood sugar of the ADH rat of −57 mg. %, which is interpreted as insulin present in the perfusate. The hyperglycemia was maintained during a second hour. The test for insulin was again positive, the change in blood sugar of the ADH rat being −103 mg. %.

A series of these observations is summarized in Table 2. Ten observations were made in which the blood sugar level in the perfusate was kept below 90 mg. %. Blood from fasted rats was used. As the perfusion proceeded, the blood sugar values continued to drop. The value for the glucose levels of the blood perfusate shown in the third column of Table 2 is the reading midway in the period. The average period for the perfusion experiment was 60 minutes. At the end of this

time, 3 cc. of the blood perfusate was centrifuged and the plasma injected into an ADH rat for assay. In every assay of these 10 experiments there was a rise in blood sugar in the test animal, the range being from +16 to +96 mg. %. According to this method of assay no insulin was present in the perfusate. In one of these experiments the perfusion was carried on for 134 minutes. The sugar level midway in the experiment was 44 mg. %. The assay for insulin at the end of the period was negative. This observation bears out our impression that insulin is not "washed out" of the pancreas by prolonged perfusion.

In another series, comprising 19 observations, glucose was added

TABLE 2. INFLUENCE OF BLOOD GLUCOSE LEVEL ON INSULIN SECRETION

Nature of experiment	No. observations	Glucose level of perfusate (mg. %) (range)	Test for insulin in perfusate	
			Change in blood glucose of ADH rat (mg. %) (range)	Interpretation
Pancreas perfused with blood low in glucose	10	84– 35	+16 to + 96	no insulin
Pancreas perfused with blood high in glucose	19	141–569	−12 to −103	insulin
Control. (Blood not used for perfusion)	19		+15 to +100	no insulin

to the perfusate, the levels ranging from 141 to 569 mg. % (Table 2). When the perfusate was assayed for insulin, usually after an hour's perfusion, in every instance the test animal showed a fall in blood sugar. The range was from −12 to −103 mg. %. This is interpreted as insulin being present in the perfusate.

Control samples of blood which had not been in the perfusion apparatus were assayed for insulin. In all of the 19 samples the test for insulin was negative.

SUMMARY AND CONCLUSION

The technique for removal of the pancreas and its maintenance in a perfusion apparatus is described.

When the pancreas is perfused with blood which has a low glucose content, insulin is not secreted in an amount detectable by the assay method used.

A high glucose level in the perfusate stimulates the islet cells directly to secrete insulin.

It is concluded from these findings that the method described for the perfusion of the pancreas is sufficiently sound within certain physiological limits to be used for testing the hormonal influences on insulin secretion.

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SUPPRESSION OF INSULIN SECRETION BY THE GROWTH HORMONE OF THE ANTERIOR PITUITARY AS DETERMINED WITH THE ISOLATED RAT PANCREAS IN A PERFUSION APPARATUS[1]

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THE DIABETES which is produced by the administration of anterior pituitary extract (APE) is the result of an interplay of several factors. These include the direct effect of APE upon 1) the pancreas, 2) other endocrine glands, especially the adrenal and thyroid, and 3) other tissues such as muscle and liver. The interaction of these various factors and their impact on the entire organism make the problem of pituitary diabetes exceedingly complex. An understanding of the separate factors is necessary before the problem as a whole can be analyzed. This paper deals with an attempt to study an isolated factor, namely the direct effect of APE upon the insulin-producing cells of the pancreas.

The question of a pancreatrophic hormone of the anterior pituitary is still unsettled. Extensive study has failed to give proof of such a hormone. Richardson and Young (1937) have shown that APE causes hypertrophy of islet tissue in rats, and Marks and Young (1940) have shown that it doubles the insulin content of the rat's pancreas after two weeks of administration. By the same method Ogilvie (1944) has demonstrated islet hypertrophy in rabbits. However, the fact that hypophysectomy is not followed by involutionary changes in the islets of Langerhans in any species (Houssay, 1937) nor by impairment in the secretory capacity of this tissue (Houssay et al., 1942; Anderson and Long, 1947a) is strong evidence against a pancreatrophic function of the pituitary. Furthermore, experimental work on dogs and cats suggests an antagonistic rather than a stimulating action of the pituitary on the pancreas. Parenteral administration of APE to dogs causes a decrease in the insulin content of the pancreas

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which is demonstrable as early as eight hours after the first injection, and the insulin content continues to fall as the daily injections of APE are continued (Best, Campbell and Haist, 1939); associated with this is an impairment in the secretory capacity of the islets (Houssay et al., 1942). Finally a "permanent" diabetes ensues as the result of insulin insufficiency (Young, 1938). Parallel with the diminishing functional capacity of the pancreas, hydropic degeneration and atrophy of the islets have been shown to occur (Richardson and Young, 1938; Ham and Haist, 1941).

In an effort to determine the nature of the direct effect of the anterior pituitary on the pancreas, we have set up experiments in which the pancreas was removed from the body, transferred to a perfusion apparatus, where it was kept in a healthy functioning state, then subjected to the influence of the anterior pituitary hormones, after which the blood which had been circulating through the pancreas was assayed for insulin.

METHODS

The perfusion apparatus employed in this study was designed and built by one of the authors (Long, 1946). The operative technique and the procedure of the perfusion experiments have been previously described (Anderson and Long, 1947b). The method for detecting insulin in the perfusate was the same as that devised by Anderson, Lindner and Sutton (1947) for the assay of insulin in blood.

EXPERIMENTAL DATA

In our earlier experiments we used crude extracts of beef anterior pituitary in the perfusate, but they proved so toxic to the pancreas that the experiments were valueless. Our first successful studies were carried out with purified growth hormone. One batch of this material was prepared by Marx (Marx, Simpson and Evans, 1943) and the other by Li (Li, Evans and Simpson, 1945). The material in each case had been shown to be free of gonadotrophic, adrenocorticotrophic, and thyrotrophic hormones. The growth hormone prepared by Marx had been shown to have diabetogenic properties in that it produced glycosuria in fed rats which had been partially depancreatized (Marx et al., 1943).

In this series of experiments, male rats of approximately 180 grams body weight were used. They had been fasted at least 17 hours, in order to reduce the insulin content of the pancreas and of the circulating blood to a basal level (Haist, 1944) and also to facilitate the operative technique by having the stomach and duodenum practically empty. The blood pressure maintained by the perfusion pump was 130 mm. Hg., and the temperature of the tissues was kept around 100° F. Under these conditions the rate of blood flow through the organs was maintained at about 2.7 cc. per minute. The rate of oxygen consumption was not measured, but a striking color difference be-

tween arterial and venous blood was taken as evidence of a satisfactory exchange of oxygen and carbon dioxide. The perfusate consisted of freshly drawn heparinized blood taken from fasted rats. The plasma used to bathe the organs in the chamber had been collected from rats similarly fasted. The total circulating volume in the apparatus was 12 to 15 cc., and the volume of plasma in the chamber 6 cc.

In the first set of experiments, the problem was to ascertain whether growth hormone acted directly upon the islet cells, stimulating them to secrete insulin; in other words to test whether the growth hormone had pancreatrophic properties. In three experiments growth

TABLE 1. INFLUENCE OF GROWTH HORMONE ON THE SECRETION OF INSULIN

	No. observations	Amount growth hormone (mg.)	Glucose level of perfusate* (mg. %)	Test for insulin in perfusate	
				Change of blood glucose in ADH rat (mg. %)	Interpretation
Section A					
Growth hormone and low glucose level	3	1.0, 1.0 0.08	54, 49 87	+60, +30 +25	no insulin
Control (no growth hormone)	10	0	84–35 (range)	+16 to +98 (range)	no insulin
Section B					
Growth hormone and high glucose level	5	0.08	240–441 (range)	+196, +80 +99, 0 +28	no insulin
Control (no growth hormone)	19	0	141–569 (range)	−12 to −103 (range)	insulin
Control (growth hormone inactivated)	1	0	281	−36, −20	insulin

* Blood glucose level of the perfusate, midway in the perfusion period.

hormone was added to the perfusate in the amounts of 1.0, 1.0 and 0.08 mg. respectively (1.0 mg. = 10 units growth hormone; Li, Evans, and Simpson, 1945). In order to prevent the stimulation of insulin secretion by hyperglycemia the glucose content of the perfusate was allowed to drop during the period of perfusion from the level of fasting blood sugar (circa 90 mg. %) to hypoglycemic levels of about 30 mg. %. After circulation through the pancreas for one hour, 3 cc. of the blood perfusate (or 1.5 cc. of the plasma) were injected into the adrenodemedullated diabetic hypophysectomized (ADH) rat for the detection of insulin. The results are shown in Section A of Table 1. In each of the three experiments the test for insulin was negative. In 10 control experiments in which no growth hormone had been added to the perfusate the test for insulin was likewise negative. One may conclude from these findings that growth hormone does not stimulate the islets directly to secrete insulin.

In another set of experiments growth hormone was studied from the standpoint of its possible inhibitory effect on insulin secretion. It had been shown previously that an elevated glucose level in the perfusate stimulates the islet cells to secrete insulin (Anderson and Long, 1947b). In this group of experiments the combined effect of growth

hormone and hyperglycemia was studied. Growth hormone in a dose level of 0.08 mg. was added to the perfusate in which the glucose level was 250 mg. % or higher. The experimental data are given in Section B of Table 1. It will be seen that the presence of growth hormone prevented the insulin secretion which would be expected to occur with an elevated blood sugar. When the growth hormone was inactivated by heating in a boiling water bath for an hour, its inhibitory effect on insulin secretion disappeared.

The interpretation of these findings, i.e. that growth hormone is inhibitory to insulin secretion, could be challenged on the basis that

TABLE 2. ASSAY OF INSULIN IN PLASMA IN PRESENCE OF GROWTH INSULIN HORMONE

Insulin milli-units	Growth hormone mg.	Change in blood glucose of ADH rat mg. %
0.5	0.2	−56
0.5 (17)*	0	−43 ±11.0
1.0	0.04	−72
1.0	0.04	−81
1.0 (17)*	0	−50 ±24.8

* Number of animals in brackets.
The insulin and growth hormone were dissolved in 1 cc. plasma.

TABLE 3. RAT ANTERIOR PITUITARY EXTRACT AND INSULIN SECRETION

	Period of perfusion minutes	No. rat pituitaries	Glucose level of perfusate mg. %	Test for insulin in perfusate	
				Change of blood glucose in ADH rat mg. %	Interpretation
Whole Pituitary	95	5	626	+21	no insulin
Anterior Pituitary	60	6	220	+20	no insulin
Anterior Pituitary	25	6	340	+40	no insulin

growth hormone present in the perfusate might antagonize any insulin present and thus obscure it in the insulin assay test. To test this possibility, known amounts of growth hormone and insulin were added to plasma and the mixture assayed for insulin. The results are shown in Table 2. It is evident from this that when growth hormone and insulin are mixed in vitro and injected intravenously, the fall in blood sugar of the ADH rat in response to the insulin is so rapid that if any antagonism exists between growth hormone and insulin it does not have time to manifest itself.

Rat pituitary extract inhibits insulin secretion in the same way as the purified growth hormone (Table 3). This extract was prepared by grinding frozen rat pituitaries and extracting with saline. Posterior pituitary principle, when present in the extract, impaired the circulation so that it was found advisable to dissect the anterior lobe free of the posterior lobe.

Thyroxin and adrenal cortical extract also were studied in an effort to determine whether they too had an inhibitory effect on insulin secretion. Houssay (1944) has shown that an excess of thyroid hormone produces glycosuria in partially depancreatized dogs, and Long, Katzin and Fry (1940) demonstrated the same for adrenal cortical hormones when administered to partially depancreatized rats. The data obtained by us in the experiments in which thyroxin and adrenal cortical extract were added to the perfusate containing a high blood sugar, are summarized in Table 4. It will be noted that neither of these hormones inhibits the stimulating effect of hyperglycemia on insulin secretion, and it may therefore be concluded that neither the thyrotrophic nor the adrenocorticotrophic hormones of the pituitary inhibits insulin secretion. The lactogenic hormone of the pituitary and the gonadal hormones were not studied.

TABLE 4. ISLET FUNCTION IN PRESENCE OF ADRENAL CORTICAL HORMONE AND THYROXIN

	No. observations	Amt. hormone in 20 cc. perfusate	Glucose level in perfusate mg.%	Test for insulin in perfusate	
				change in blood glucose in ADH rat mg.%	Interpretation
Perfusion with adrenal cortical hormone* and high glucose level	4	0.04 cc	343–440 (range)	−60, −21, −24, −36	insulin
Perfusion with thyroxin and high glucose level	2	0.01 mg	192, 228	−36, −18	insulin

* Upjohn's adrenal cortical extract.

DISCUSSION

The experimental data reported here do not support the concept of a pancreatrophic hormone of the pituitary, but rather the reverse, namely, that the anterior pituitary contains a factor which inhibits insulin secretion. The observation that inhibition is obtained with a purified preparation of growth hormone and is not obtained with either adrenal cortical hormone or thyroxin suggests that it is the growth hormone rather than the adrenocorticotrophic or thyrotrophic hormone which acts directly upon the islet cells. This does not exclude the adrenocorticotrophic and thyrotrophic hormones as diabetogenic factors acting in some manner on extrapancreatic tissues.

SUMMARY AND CONCLUSIONS

The experimental work consisted of the removal of the pancreas from the body, and while maintaining it in a healthy functioning state in a small perfusion apparatus, subjecting it to the influence of the hormones of the anterior pituitary, the thyroid and the adrenal.

Growth hormone of the anterior pituitary does not stimulate the islets directly to secrete insulin.

Rat anterior pituitary extracts and growth hormone inhibit the secretion of insulin which occurs in response to a high level of blood glucose.

When growth hormone and insulin are mixed in vitro and injected intravenously, the effect of insulin on the blood sugar level occurs too rapidly for the growth hormone to show an anti-insulin effect.

Thyroxin and adrenal cortical hormone do not inhibit the secretion of insulin.

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THE ESSENTIAL LESION IN EXPERIMENTAL DIABETES INSIPIDUS

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WE HAVE stated (1941) that the von Hann view that diabetes insipidus (DI) cannot occur in the absence of pars distalis is incorrect, since sustained DI occurred in dogs as a result of total hypophysectomy, and persisted after removal of the pars distalis where DI had been produced by denervation of the neurohypophysis, complete absence of pars distalis being proved by serial sections. The present report confirms and extends these findings, offers an explanation for the apparent discrepancies in other workers' findings, and reports further observations on other endocrine influences on renal water output.

Permanent DI resulting from total hypophysectomy. Methods are described in an earlier communication (Heinbecker and White, 1941). Total hypophysectomy, including destruction of median eminence, was performed through the oral approach on a series of dogs under sodium pentobarbital anesthesia. Fifteen developed marked sustained (8 to 20 × normal output) DI, were followed for many weeks or months, up to 30 months, and were shown at autopsy to have no pars distalis remaining (serial sections of hypothalamus and of sellar contents or of decalcified sella with contents). Two others developed similar polyurias but were found at autopsy to have 5 per cent or less of pars distalis remaining. Five others were shown to be totally hypophysectomized (all divisions of hypophysis absent) but did not sustain their polyurias; these animals showed infection, with abscesses in the hypothalamus or distention of third ventricle, and loss of appetite and weight. In the remaining 19 animals autopsy showed that hypophysectomy had not been total. Sixteen showed complete absence of pars distalis but with remaining fragments of neurohypophysis (median eminence) and a residuum of supraoptic nucleus; none of these developed a high sustained polyuria, although mild DI (1.5 to 4 × normal) was often seen. The other 3 showed remaining fragments both of pars distalis and of neurohypophysis; polyuria was inconstant and never maximum.

Figures 1 and 2 show the results on 2 groups of proven totally hypophysectomized dogs, figure 2 being of longer duration. Comparison with figure 3, of 3 "puncture" dogs (denervation of neurohypoph-

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ysis, pars distalis remaining) shows that the polyuria is not so well sustained in the absence of pars distalis, although the decline may not be evident until months have elapsed; the output always remains at several times the normal. The normal interphase never appeared

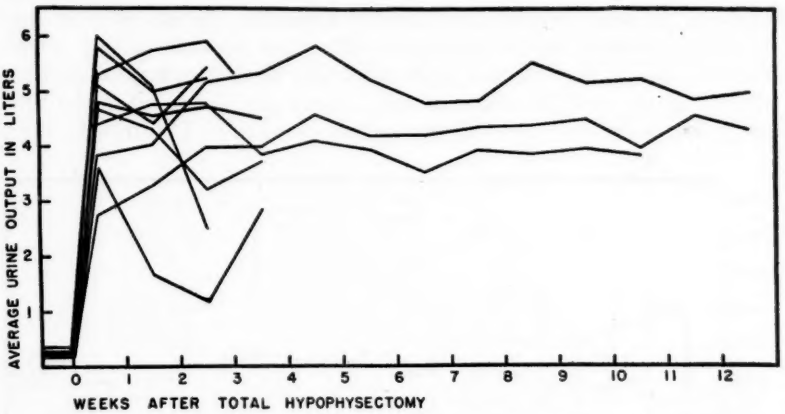

FIG. 1. Average daily urine outputs in weekly periods of 10 totally hypophysectomized dogs. The animal showing the drop between first and third weeks showed weakness and loss of appetite during this period; the experiment was terminated after recovery in fourth week.

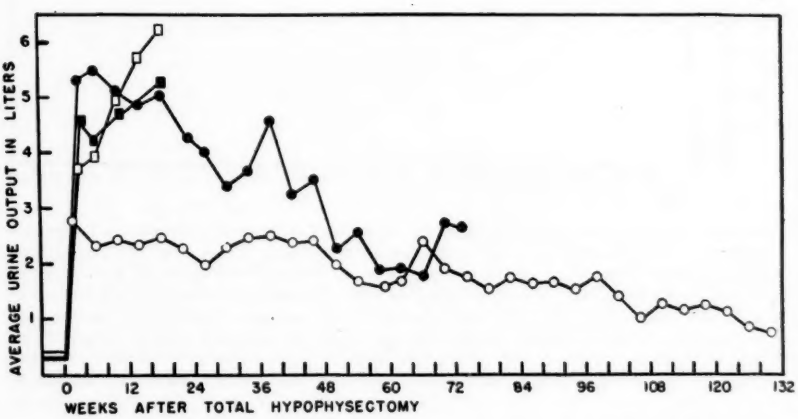

FIG. 2. Average daily urine outputs in four week periods of 4 totally hypophysectomized dogs.

in these dogs, where all pitressin-forming tissue was removed at one sitting. This would appear to eliminate any postulated secretion of hypothalamic nuclei as causing the normal interphase when it is seen, as after denervation of neurohypophysis, since these nuclei were in-

tact in the present series of 15 dogs at the time the normal interphase would have appeared.

Effect of removing the pars distalis from dogs previously rendered polyuric through denervation of neurohypophysis. In 3 cases, after polyuria had been produced by deneravtion of neurohypophysis, the pars distalis was removed at a second operation. In no instance was there an abolition of the polyuria; rather, an increase might occur. The reason for the increase seen after the second operation is that some fragments of pitressin-forming tissue, remaining after the first operation, were destroyed at the second operation. The results, seen

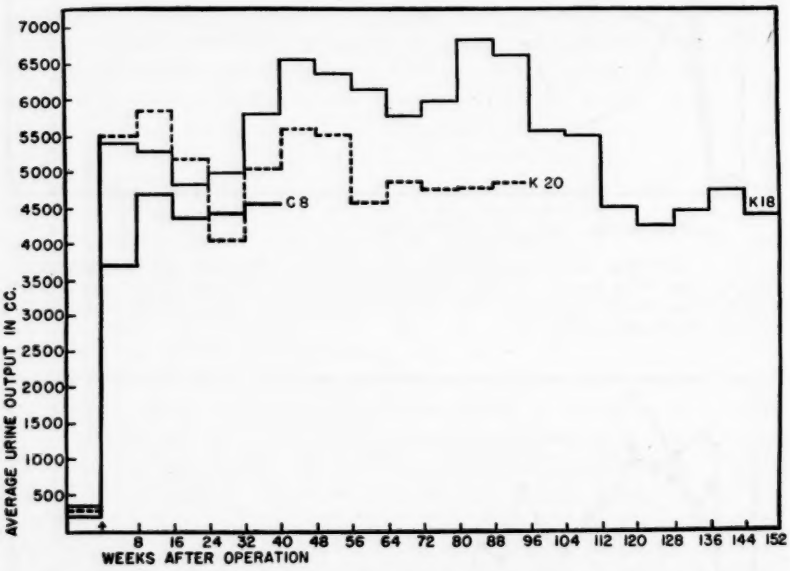

FIG. 3. Average daily urine outputs in eight week periods of 3 "puncture" dogs, denervation of neurohypophysis with pars distalis remaining. These dogs showed normal interphase during second week; time scale does not permit their showing in this figure, and those days have been omitted from averages. Polyuria is sustained longer than in total hypophysectomy, although with the latter the polyuria for several months may be as high as with the puncture dogs. The polyurias of successful punctures are maintained *permanently;* K18 of this figure has been followed three years longer than the figure is carried, maintaining a daily output of 5 to 6 liters for six years after puncture.

in figure 4, are in accord with those of figures 1 and 2 in showing that pars distalis is not essential to a marked, although not necessarily maximally sustained, polyuria. We believe that the findings of other workers that DI is immediately abolished by removal of pars distalis were on animals with sufficient residuum of neurohypophysis to be incompatible with DI in the absence of pars distalis, while permitting DI in the presence of pars distalis.

Median eminence. Rioch, Wislocki and O'Leary (1940) illustrate the median eminence of the monkey (their figure 21A) and of the cat (21B) as extending in median sagittal section backward to the angle formed with the floor of the premamillary recess and forward to or almost to the optic chiasma. Most workers in this field are agreed that the median eminence, where defined "as constituting the bulbous part of the infundibulum down to the point where the straight infundibular stem clearly commences" (Rioch, et al., 1940, p. 26), constitutes an

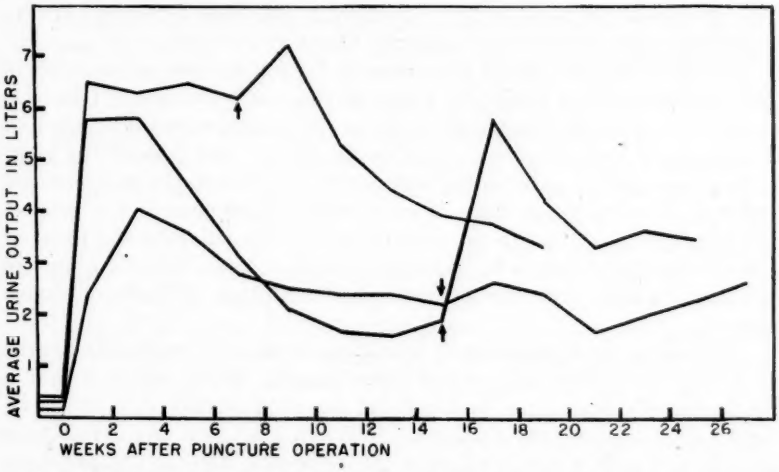

FIG. 4. Daily urine outputs in two week periods of 3 dogs where total hypophysectomy followed puncture. Total hypophysectomy at arrows; no diminution of polyuria resulted except after four weeks in upper curve.

important division of the neurohypophysis. It has been estimated as contributing 13 to 20 per cent of the neurohypophysis in the monkey, based on volume measurements and on counts of the residuum of normal supraoptic nucleus cells after section of the infunidbular stem (Magoun and Ranson, 1939; Magoun, Fisher and Ranson, 1939), and 10 to 20 per cent in the rat, dog, monkey and man, based on supraoptic nucleus residuum after section of stem (Rasmussen, 1940). This conclusion is not affected, regardless of the merits of the finding of Weaver and Bucy (1940) that the rostral limits of the median eminence stop short of the chiasma.

The pars distalis in DI. It is almost universally accepted that destruction or denervation of the neurohypophysis is of primary importance in the pathogenesis of DI. Uncertainty has persisted as to the degree to which such destruction or denervation must be carried to make the DI maximum and permanent; there has remained also the question as to whether or not presence of pars distalis is essential for a state of DI.

The evidence that in 15 dogs it has been possible to maintain marked DI for many months without any pars distalis would appear crucial, and adequate to prove that an active pars distalis is not essential. These dogs, however, differ from dogs with remaining pars distalis in that the polyuria begins to diminish after several weeks or months, dropping to 4 to 6 times the normal output by 12 to 18 months, with a still further decline by 30 months (fig. 2). In this respect we revise our earlier statement (1941) that loss of pars distalis has no effect on the polyuria produced by loss of neurohypophysis; the decline is revealed only by following the dogs for long periods, and the output remains permanently above the normal.

In those dogs in which a maximum DI follows loss of function of the supraoptic and paraventricular nuclei, we have found that this must be practically complete as far as the paired supraoptic nucleus is concerned and it must include 30 to 50 per cent loss of the large cells of the paired paraventricular nucleus, particularly in its rostral portion. A residuum in dogs of even about 5 per cent of the cells of the supraoptic nucleus in the absence of any pars distalis will prevent the existence of a state of maximum DI, while a residuum of about 15 per cent in such dogs will prevent any exhibition of increased urine output.

Studies of the hypothalami from dogs in which a moderate degree of DI, 3 to 6 times the normal urine output, exists indicate that a residuum of supraoptic and rostral paraventircular cells may be large enough to prevent a state of DI in a dog without any active pars distalis and still permit moderate DI to be exhibited in the presence of the pars distalis. Supporting evidence for this interpretation is afforded by the observation that in simple hypophysectomy dogs without DI the giving of anterior lobe extract will increase the urine output 3 to 6 times.

It has been noted also that a state of maximum DI is maintained indefinitely in dogs without any active neurohypophysis, but with the pars distalis left intact. When the pars distalis is removed from such dogs at a subsequent operation, there occurs, after a variable lag, a slow, gradual decrease in urine output. Evidence that this decrease may be attributed to a gradual atrophy of other endocrine glands, as adrenal cortex and thyroid, which are normally under the influence of the pars distalis has been secured.

While we cannot present strictly quantitative data, the results of microscopic studies of many incompletely hypophysectomized dogs are compatible with the view that there is some critical fraction of residual functional neurohypophysis (in the dog probably 5 to 10 per cent) which suffices to prevent development of a frank DI in the absence of pars distalis but which is compatible with a moderate to marked DI in the presence of a considerable fraction of pars distalis. In the *complete* absence of functional neurohypophysis (or perhaps

with a residuum of less than 3 per cent) a marked DI is exhibited in the dog even in the complete absence of pars distalis, although the urine output will usually decline somewhat after many weeks or months. This concept is consistent with all our findings in the dog and, we believe, with those of other workers, on other species as well as on the dog. There is apparently some species difference in that even the highest recorded urine outputs in the cat and monkey are proportionally lower than in the rat, dog and man, but this does not affect the above concept.

Figure 5, C and D, shows that complete destruction of the neurohypophysis results in complete disappearance of the supraoptic nucleus; this totally hypophysectomized dog had a daily urine output of 4 to 6 liters for many weeks. Figure 5, E and F, shows the residuum of supraoptic nucleus after "simple" hypophysectomy, which in this case included partial encroachment on the median eminence. This dog showed very mild DI, the daily urine output ranging from 1 to 2 × normal. This extent of residuum of supraoptic nucleus and neurohypophysis will often result in a polyuria of 3 to 6 × normal output when all or a considerable fraction of pars distalis remains.

Other endocrine influences on renal water output. A similar routine was followed twice in one totally hypophysectomized dog and once in a second such dog, with similar results in all instances. The objective was to produce an excess of the adrenal cortical and thyroid hormones known to increase urine output and to determine whether in the presence of such excess the administration of anterior lobe extract would have any further diuretic effect. Adrenal cortical extract,[2] 8 cc. per day subcutaneously, was first administered, with no significant change in water exchange. After a short interval, desoxycorticosterone, 6 mgm. per day subcutaneously, was given, with slight increase in urine output. While continuing desoxycorticosterone, thyroid, 0.1 gm. per kilo per day by mouth, was added, with a large increase in urine output. Then, while continuing both desoxycorticosterone and thyroid, sheep anterior pituitary extract,[2] 225 units per day subcutaneously, was added. This resulted in a definite decrease in urine output throughout the period of anterior lobe administration. The results are seen in figure 6; water intake was followed but is not shown in figure; it closely paralleled urine volume, with no evidence of water retention or of dehydration through any of the extracts. Since a single daily dose of the anterior lobe extract kept the urine output at a lower level for 24 hours, it is improbable that the decrease could be due to contamination with posterior lobe extract.

[2] The Preloban was generously furnished by the Winthrop Chemical Company. The manufacturer's note states that it contains a somewhat varying amount of the thyrotropic factor, and of the lactogenic factor, possibly traces of other adenotropic factors, and a standardized quantity of gonadotropic hormone, where the hypophysectomized rat was the assay animal. It is stated to contain no posterior lobe hormone. The adrenal cortical extract was Upjohn's.

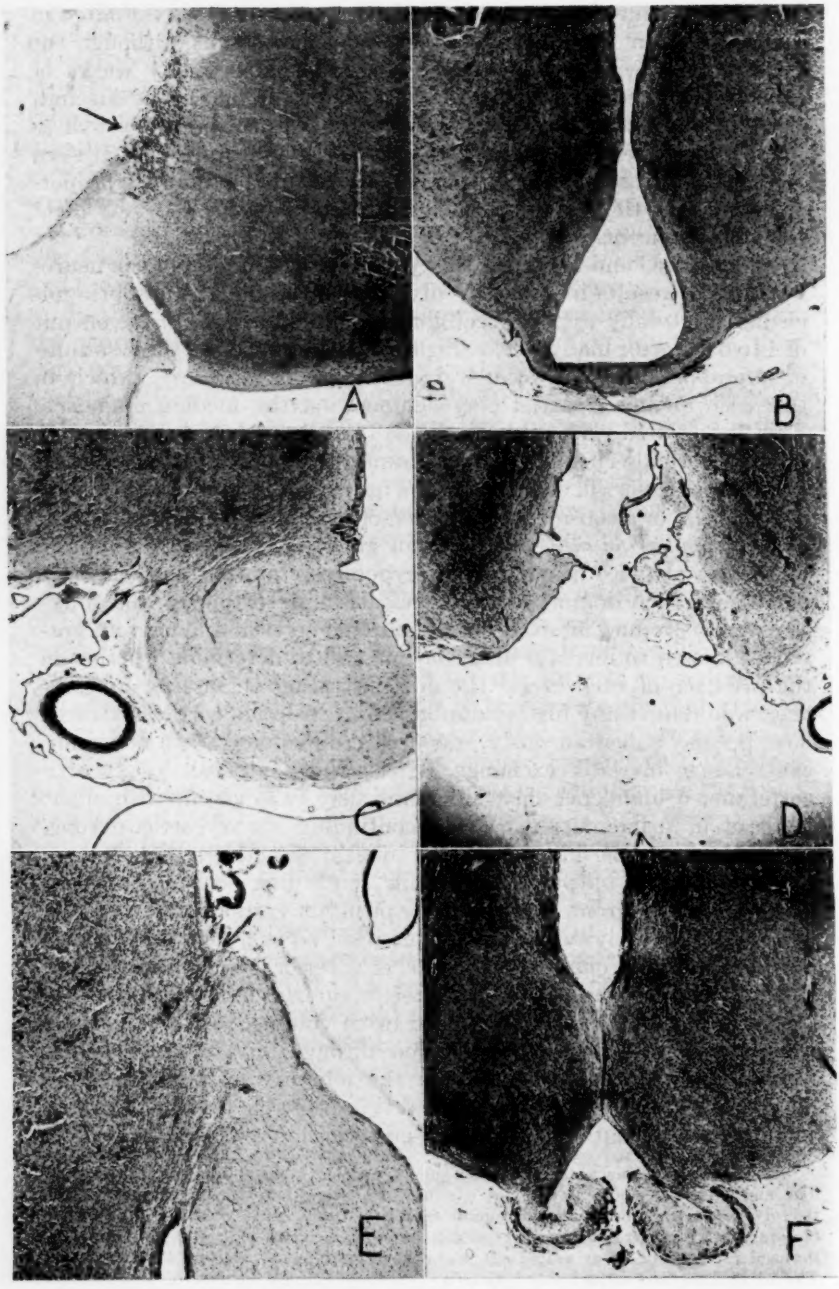

FIG. 5. See next page for description.

From these results it is concluded that in the presence of an excess of adrenal cortical and thyroid hormones, the action of anterior lobe extract is antidiuretic and that the diuretic effect usually seen in the hypophysectomized dog depends upon the trophic action on the adrenal cortex and thyroid. The gradual decline in the urine output of the totally hypophysectomized dog is explained on the basis of

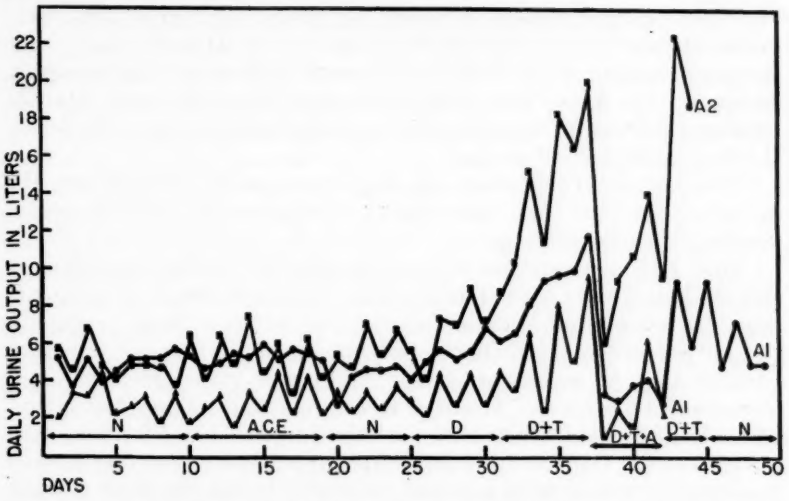

FIG. 6. Daily urine outputs in 2 experiments on total hypophysectomy dog A1, and 1 experiment on total hypophysectomy dog A2.
●—● observations on A1 begun 18 days after total hypophysectomy;
▲—▲ observations on A1 begun 9 months after total hypophysectomy;
■—■ observations on A2 begun 3 months after total hypophysectomy.
N designates period of no extract administration, A.C.E. adrenal cortical extract 8 cc. per day subcutaneously, D desoxycorticoesterone acetate 6 mgm. per day subcutaneously, T thyroid 0.1 gm. per kilo per day orally, A anterior lobe extract 225 units per day subcutaneously.

diminishing adrenal cortical and thyroid function; histological studies of the thyroids and adrenals of hypophysectomized dogs have shown progressive atrophy even up to 2 years (Heinbecker, White and Rolf, 1944). These observations are regarded also as indicating why in the

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FIG. 5. A. Supraoptic nucleus of normal dog, rostral portion.
B. Median eminence of same dog.
C. Area of supraoptic nucleus of totally hypophysectomized dog. All cells of the nucleus on this and on the other side failed to stain. Marked sustained DI exhibited.
D. Area of median eminence of same dog as in C.
E. Residual cells of supraoptic nucleus in a "simple" hypophysectomy dog; other side showed no cells. Urine output 1 to 2× normal.
F. Median eminence of dog as in E; complete loss of supraoptic cells on damaged side; nucleus of undamaged median eminence side shown in E. Simple hypophysectomy does not typically encroach on median eminence.

puncture and in the normal dog, and in the monkey, a decrease in urine output frequently is a first result of the administration of anterior lobe extract; only later, if at all, does the diuresis follow (White and Heinbecker, 1937).

The effects of thyroidectomy and of thyroid administration on urine output. In 2 totally hypophysectomized DI dogs subsequent thyroidectomy (7 weeks and 17 days later) did not affect or slightly lowered the urine output within a week; unfortunately, these dogs were followed only a week after thyroidectomy. Dog 24 had a daily urine output averaging 4.5 to 5 liters for 7 weeks following total hypophysectomy; this figure was unchanged after thyroidectomy. Dog 31 averaged 5 liters for 17 days after total hypophysectomy; this fell to 3.5 liters after thyroidectomy.

In 3 simple hypophysectomy dogs (median eminence intact) the transitory polyuria was immediately abolished by thyroidectomy, confirming earlier findings.

Oral administration of 0.1 gm. desiccated thyroid per kilo per day for 14 days to 3 normal dogs had no significant effect on urine output. The same administration caused a small percentage increase in the output of 2 dogs rendered polyuric by hypothalamic puncture (denervation of neurohypophysis); K18 was raised from a daily average of 6.6 liters to 7.1, with a return to 5.2 after cessation of thyroid administration, while K20 was raised from 4.1 to 5.9 liters, with a return to 4.7. One total and one simple hypophysectomy dog showed increases of 70 and of 26 per cent, respectively, on the same thyroid administration. These findings are consistent with those of other workers on the dog and cat, that thyroid administration has a greater effect on urine output in the absence or with a deficit of neurohypophysis.

SUMMARY

Experimental diabetes insipidus (DI) in dogs follows destruction or denervation of the entire neurohypophysis; all the cells of the paired supraoptic nuclei and the rostral half to third of the paired paraventricular nuclei innervate the neurohypophysis.

The presence of pars distalis is not essential to a marked DI lasting many months but is essential to its permanent maximum maintenance through trophic effects on thyroid and adrenal cortex.

Moderate DI may exist in the presence of the pars distalis when the residuum of functional neurohypophysis is sufficient to prevent any increase in urine output in the absence of the pars distalis.

When sufficient exogenous thyroid and desoxycorticosterone are administered simultaneously to produce a marked increase in urine output of the totally hypophysectomized DI dog, the additional administration of anterior lobe extract decreases the urine output.

Thyroidectomy immediately terminates the transitory polyuria of simple hypophysectomy. It will not eliminate but may depress

somewhat the polyuria of the totally hypophysectomized dog (complete absence of all divisions of hypophysis).

Oral administration of desiccated thyroid 0.1 gm. per kilo per day for 14 days does not materially alter the urine output in normal dogs; in simple and in totally hypophysectomized dogs and in dogs rendered polyuric through denervation of neurohypophysis it somewhat increases urine output.

The results of other investigators on the essential lesion of DI, in other mammals as well as in the dog, conform to those reported here, on the premise that failure of complete destruction or denervation of the neurohypophysis, including the median eminence, may prevent development of DI in the absence of pars distalis, while the presence of pars distalis may permit the development of DI even in the presence of a slight residuum of functional neurohypophysis; evidence for this premise is presented.

The term "total hypophysectomy" should be restricted to those operations where all divisions of the hypophysis, including median eminence and stalk, have been removed or rendered functionless. Procedures short of this but including removal of pars distalis should be termed "simple hypophysectomy"; if the unqualified term "hypophysectomy" is employed, specific reference as to its degree of completeness should be made.

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NOTES AND COMMENTS

EFFECT OF THIOURACIL ON OXIDATION OF PARAPHENYLENDIAMINE BY VARIOUS TISSUES

THE MECHANISM by which thiourea derivatives and other antithyroid drugs inhibit formation of thyroid hormone is as yet not known. It is generally assumed that at least certain steps in the synthesis of thyroid hormone are controlled by enzyme action. The influence of thiourea derivatives on various enzyme systems has been reported. It has been suggested also that thiourea derivatives may react with iodine, predominantly in their capacity as reducing agents, and that this may constitute the basis for their antithyroid action.

We have previously reported that the cytochrome-cytochrome-oxidase system of the thyroid gland is inhibited by thiouracil in vivo and in vitro

TABLE 1. INHIBITORY ACTION OF THIOURACIL (0.002 M) ON PARAPHENYLENDIAMINE OXIDASE BY RAT TISSUES

Exp. #	No. of exp.	Tissue	Oxidase index (per mg. tissue)			P of difference
			Control M	Experimental M	Difference	
1	11	Thyroid	35.9	16.9	−19.0	<0.01
2	21	Bone Marrow	28.3	27.7	− 0.6	>0.8
3	8	Liver	32.9	29.3	− 3.6	>0.3
5	5	Kidney	93.8	90.7	− 3.1	>0.8
5	10	Brain	28.4	25.0	− 3.4	>0.3

(Paschkis, Cantarow and Tillson, 1945). Since this system occurs ubiquitously, it seemed of interest to extend these studies to other tissues. The present report deals with the influence of thiouracil on oxidation of paraphenylendiamine by bone marrow, liver, kidney and brain in vitro.

Methods. All studies were made on tissue brei of adult white rats of both sexes. Bone marrow was taken from the femur. The technique employed has been described previously.

Results. The pertinent data are summarized in Table 1. Data for thyroid are summarized from our previous report. Oxidation of paraphenylendiamine by bone marrow, liver, kidney, and brain was not inhibited by thiouracil in the same concentration which significantly inhibited its oxidation by thyroid tissue.

Discussion. Our previous studies indicated that the thyroid glands of rats treated with thiouracil for 11 to 21 days had significantly lower capacity for oxidizing paraphenylendiamine than did normal controls. Furthermore, addition of thiouracil inhibited oxidation of paraphenylendiamine by thyroid brei significantly. The same results in vitro were obtained

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with a number of other inhibiting compounds. McShan, Meyer and Johansson, (1946) subsequently showed that the lack of color observed upon addition of ascorbic acid was due to decoloration of the blue oxidation product of paraphenylendiamine rather than to inhibition of the enzyme system. Glutathione was found by these authors to inhibit cytochrome oxidase to the extent of 50% and, in addition, decolorized the oxidation product formed by the remaining 50% of the enzyme. However, thiourea, thiouracil and cysteine failed to decolorize the blue oxidation product.

Interpretation of the inhibitory effect of sulfonamides and thiouracil upon paraphenylendiamine oxidation by thyroid is complicated by the fact that in experiments with thyroid slices using the manometric Warburg technique Lerner and Chaikoff (1945) found no inhibition of cytochrome oxidase by thiourea and thiouracil, and only slight inhibition by sulfonamides, McShan, Meyer and Johansson, (1946), working with homogenized thyroid tissue and using an ascorbic acid substrate reinforced by Cytochrome C, found slight decrease of QO_2 by thiourea and none by sulfonamides and thiouracil.

The discrepancy between the essentially negative results of the manometric studies of Lerner and Chaikoff and of McShan *et al.* on the one hand, and our positive findings with paraphenylendiamine cannot be explained at present. That the inhibitory action of thiouracil upon the oxidation of paraphenylendiamine by thyroid is not merely the result of the chemical properties of the reagent, is suggested by the failure of thiouracil in the same concentration to inhibit oxidation induced by tissues other than the thyroid.

It is unlikely that the oxidizing enzyme system in the thyroid inhibited by thiouracil is specific for this organ. The fact that no inhibition was obtained in the tissues other than the thyroid raises the question as to whether there are substances protecting the enzyme system in these tissues, or substances sensitizing the enzyme system to thiouracil action in the thyroid. Preliminary experiments have failed to reveal evidence to support either of these hypothesis.

The lack of effect of thiouracil on the bone marrow is particularly interesting because of the occurrence of agranulocytosis in patients treated with thiouracil. Warren (1945) has obtained inhibition of rabbit bone marrow respiration with thiouracil in much higher concentrations than those employed in our experiments. He also found that myeloid marrow produced by pretreating the rabbits with croton oil, is more sensitive than normal marrow to the inhibiting action of thiouracil. The difference in results obtained by Warren and by us is probably due to the fact that we used normal marrow and employed thiouracil in the same low concentration which inhibited the oxidase activity of thyroid tissue. Whether there is a species difference in this regard cannot be stated.

Summary. Thiouracil in 0.002 M concentration inhibits oxidation of paraphenylendiamine by rat thyroid tissue brei (cytochrome oxidase system). In the same concentration thiouracil failed to inhibit oxidation of paraphenylendiamine by bone marrow, liver, kidney and brain (rat).

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INCREASED PITUITARY AND ADRENAL WEIGHT PRODUCED BY ESTRONE IN INTACT AND CASTRATED NORMAL AND "RUNT" RATS

IN A NOTE to this JOURNAL, Simpson and Williams (1946) stated that they had been unable to confirm Smith's finding (1944) that the pituitary glands of castrated mature male rats showed an increase in weight from the injection of smaller amounts of estrone than were required to produce the same effect in intact mature males. These authors misquoted the original investigators in that in neither of the articles referred to (Smith, 1944, and Smith and Smith, 1944) was it stated that a total dose of 10 μg. of estrone over 5 days caused a significant increase in the pituitary weights of castrated animals. In the Smiths' experiments, the adrenals as well as pituitaries were weighed and also the transplantable gonadotropic activity of the pituitaries was measured upon immature female rats. It was found that the pituitary transplants gave the most sensitive index of effect upon the pituitaries, whereas pituitary weights (the only measurement made by Simpson and Williams) gave the least. Although 10 μg. of estrone caused a significant decrease in transplantable gonadotropic activity of the pituitaries of castrated males and a barely significant enlargement of the adrenals, 50 μg. were required to bring about a significant increase in pituitary weight. In intact mature males, 120 μg. gave no significant response by any of these criteria. The next larger dosage tried on intact animals was 500 μg., which gave the complete pituitary reaction. Inasmuch as this difference between castrated and intact rats constitutes part of the evidence presented by the Smiths that oxidation products of estrone (whose formation is inhibited by the presence of the testes) rather than estrone itself stimulate pituitary secretion, it seemed important that this matter be settled. Since the original publication, a large number of unpublished findings have accumulated. The results are so very consistent that there would seem to be no question as to the validity of the original observation.

In their first group of experiments the largest dose of estrone given to castrated rats by Simpson and Williams was 25 μg./100 gm. In Smith's experiments 50 μg. were required to give significant enlargement of the pituitaries of castrates. In their second group of experiments, however, their findings disagreed with Smith's in that they got a significant increase in pituitary weights in the group of intact rats that received 100 μg./100 gm. and failed to find any significant increase in the group of castrated animals that received 50 μg /100 gm. As stated in Smith's first paper (1944), all animals used for test were between 70 and 100 days old and weighed between 150 and 200 grams. Simpson and Williams failed to state the age of their

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animals (other than that they were adult), but the weights given in the second group of experiments were only 100 to 120 grams. This might, of course, be due to a difference in the strain of rats, but it seemed possible that English rats, during these difficult years, might be suffering from malnutrition. With Smith's criteria, many rats that are either too small for their age or whose weights do not match those of littermates have to be discarded. With a batch of such animals this possibility has been recently investigated. In these experiments, instead of using a control littermate of the same weight for each injected animal and expressing results in terms of percentage change *vs.* uninjected partner, the method employed by Simpson and Williams was

TABLE 1. COMPARATIVE EFFECT OF ESTRONE UPON NORMAL VS. "RUNT" 90–120 DAY OLD MALE RATS

(4 rats per group)

A. Rats weighing 150–200 gm. before injections. Normal

Intact Rats			Castrated Rats		
Total dose of estrone μg./100 gm.	Organ weights mg./kg. $\pm\sigma m$		Total dose of estrone μg./100 gm.	Organ weights mg./kg. $\pm\sigma m$	
	Adrenals	Pituitary		Adrenals	Pituitary
0	148 ±29	35 ±3	0	152 ±16	32 ±2
100	151 ±33	36 ±3	30	217 ±26	42 ±3
Difference*	+ 6 ±2.6	+ 1 ±2.2	Difference	+ 64 ±17	+10 ±1.7

B. Rats weighing 100–130 gm. before injections. "Runts"

0	162 ±16	35 ±3	0	198 ±16	43 ±7
100	274 ±73	50 ±3	30	208 ±16	43 ±9
Difference*	+107 ±86	+13 ±1.4	Difference	+10 ±21	+0.6 ±3.5

* When each injected rat is compared with a control of the same weight ±5 gm.

followed, the dosage being adjusted to μg. per 100 gm. of body weight and organ weights expressed in mg./kg. The total dose was given in 10 injections over 5 days in olive oil, 0.2 ml. per injection. Castration was performed 16 hours before injections were started and all animals were autopsied 16 hours after the last injection. The results are given in Table 1. It is apparent that the "runts" differed markedly from the fully developed animals. The intact "runts" showed a significant increase in both adrenal and pituitary weights after a dosage of estrone (100 μg./kg.) which was ineffective in the intact normal group,[1] whereas the castrated "runts" failed to respond to the dosage (30 μg./kg.) which caused significant enlargement of both glands in the castrated normals. Aside from offering a possible explanation for the findings of Simpson and Williams, the data presented are of considerable interest in themselves. The 100 μg. dosage of estrone caused a loss of weight in the intact "runts" but not in the normally developed intact animals. If organ weights had been expressed in mg./kg. of weight at autopsy rather

[1] If the adrenal weights of each injected animal of the group of intact "runts" were compared with a control of the same weight ±5 gm., the percentage increase was inversely proportional to the size of the animal, varying from 17 per cent in the 130 gm. rat to 130 per cent in the 100 gm.

than before injections, the difference between these two groups would have been even more pronounced. Furthermore, the weights of the adrenals of the castrated control "runts" were significantly greater than those of the castrated control normals and the "runts" lost weight following castration, whereas the control normals gained. It seems possible that the normal pituitary response in these underdeveloped rats was masked by a greater sensitivity to the toxicity of estrone (in the intact group) and to the trauma of operation (in the castrates). Adrenal enlargement through pituitary stimulation is a well recognized response to "noxious" stimuli.

Whether or not the observation of Simpson and Williams may be explained by the data presented, the original finding (Smith, 1944) that castration increases the sensitivity of the normal male rat's pituitary to estrone appears to be confirmed, even though the original procedure was considerably altered in this investigation.

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NEW BOOK

HARVEY CUSHING, A Biography. By John F. Fulton. Published by Charles C Thomas, Springfield, Illinois 1946. $5.00. vii+754 pp.

Dr. Fulton has outdone his hero in one respect at least; he has written a shorter, more readable biography of Harvey Cushing than Harvey Cushing did of Osler. In a way he has been aided by his subject's almost compulsive accumulation of every conceivable sort of personal record. But the job of fishing in this boundless sea of documents, notes, letters, and journals must have been enormous. For every incident in Harvey Cushing's life Dr. Fulton's book seems to have brought up just the right specimen with which to illustrate, define or ornament his evolving story. Of course he has been markedly helped by the fact that Dr. Cushing consciously tried, even in his briefest letters, to develop and maintain a high standard of prose composition. Nevertheless, an almost uncanny ear has succeeded in selecting from Cushing's many notes and addresses just those paragraphs which express most gracefully the most significant ideas. Great care has been given as well to the selection of illustrations, the problem of indexing and the composition of informative running heads for each page. Charles C Thomas who as Dr. Cushing's publisher, learned many lessons in details of format and reproduction has brought all his skill to the design and publication of the book itself. From the purely formal standpoint, there is no question but that this is work of the first class.

There is more room for debate in considering problems of emphasis and evaluation. The record has been compiled by an affectionate pupil and loyal literary heir. As such, it deals largely with the outward and visible signs of Cushing's greatness and gives only rare and possibly inadequate glimpses of more intimate affairs. With these understandable qualifications the picture seems, on the whole, an accurate one. The alert reader, or perhaps the prepared mind, will discover evidence that not all Harvey Cushing's associates were exclusively impressed by his undeniable, though somewhat episodic, personal charm. The rigors of the operating room and his compelling drive toward the lonely pinnacles of ambition sometimes resulted in words or actions which later became the subject of regret or withdrawal. Some readers may find the treatment of these painful themes a trifle muted, but they are not suppressed.

There has always been a certain measure of doubt as to Cushing's capacities as a scientist. The view adopted in this book may be a little uncertain, but on balance it appears that Harvey Cushing, though not a scientist himself, activated in one way or another a great deal of scientific work. On the technical side, of course, his contribution was enormous, and his early removals of the pituitary gland resulted in standard procedures and provocative hypotheses which formed the starting point for much modern endocrinology. Nevertheless, as this biography makes clear, there was an *ex cathedra* quality about the work which was not truly scientific. A surgeon must every day in his clinical work take positions from which there is no

retreat. Small wonder that once committed, Harvey Cushing sometimes found it difficult finally to admit that his bolder speculations would not stand critical examination.

It is more difficult perhaps to sympathize with his constant sniping at the genuine scientists in other departments. At times he seemed really to believe that medical students could better learn the basic scientific principles of medicine from the busy and preoccupied clinician than from physiologists or bacteriologists of the stature of Cannon and Zinsser.

But these matters are in a sense irrelevant. One can hardly finish the book without feeling that Harvey Cushing was, after all, a hero, or at least one of the most reasonable facsimiles we are likely to find in these realistic times. The profession of neurosurgery would have been very different without him. Indeed his heroic quality is nowhere better demonstrated than in the fact that his students have banded together to honor his ideals and practices in a society which bears his name. American surgery, in general, owes much of its tone and distinction to his example. But his scholarship and his devotion to his patients' welfare transcended party lines, as was recognized by the Royal College of Physicians when they conferred upon him the only honorary membership ever given to a surgeon.

One can't help wondering what it was that drove him so relentlessly to his unusual goal. Certainly the heroic ideal was not a fashionable one during much of the active period of his life. A psychodynamic interpretation is not possible on the evidence given us in this book, and certainly Harvey Cushing, with his antipathy to Freud, would have hated the attempt. We are told that he suffered from depression and self-distrust during his early years and again for a few months toward the end of his life. With a good many successful Americans he shared a background of Calvinism, that "vigorous, ascetic, scowling creed," which may have left him with a sense of original sin to expiate with some work of noble note. But speculation on this plane is idle.

In the more traditional analysis that Dr. Fulton gives us, certain attributes which carried Harvey Cushing to greatness are obvious. It is clear, for example, that he had an unusual, almost an overwhelming ambition. In spite of very severe physical handicaps (from the war years on he suffered from an obscure affliction of the arteries of his legs which left both femorals completely occluded), his physical and nervous energy never let him down. His mind was certainly more than ordinarily good; he had an excellent memory, an ability to learn from experience and sufficient originality not only to create a new surgical specialty but, when called upon to give an Ether Day address, to confound Back Bay with a detailed analysis of the contribution of certain dieners, biddies and handymen to the eminence of the Massachusetts General Hospital. But he was not a reflective person. He seems to have accepted the standards current in the late 19th century not only without question but with uncompromising and sentimental enthusiasm. The romanticism which led him as a youth to place a high value on membership in Scroll & Keys never left him.[1] His two major Y's, one earned in the outfield of the baseball team in 1889, and the other "in life," were probably important factors in his final return to Yale in 1933. This same

[1] It is interesting that in this he seems to have differed only in detail from his mentor, "Popsy" Welch, who felt that the only real reason for going to Yale was the chance one might have for belonging to Skull & Bones.

romantic attachment to the hero's way is also revealed in the care which he gave to the arrangement of testimonial dinners and the preparation of commemorative addresses, both activities being models of their kind. Conversely, he was appalled by the realistic school of literature which has done so much to inform us of the mechanics of human behavior and destroy its grandeur.

There are many good reasons for reading this book, not the least of them being the skilled craftsmanship of its author. It gives the story of the development of a dramatic surgical specialty, provides many illuminating sidelights on the evolution of American medical education, and, for those who appreciate the amenities of book-collecting, contains many pleasant bibliophilic anecdotes. But those who reached maturity in the troubled era which followed the First World War may well ponder the life of Harvey Cushing for the light it throws on the utility of illusions. Harvey Cushing, the product of 19th century romanticism and hero worship, was an extremely effective contributor to human welfare. Arrowsmith (Harvey Cushing's bête noire, by the way), was in many respects the beau ideal of 20th century realism. But he didn't really live and certainly wasn't very effective.

But before this review gets lost in the misty paradoxes of being and not being, let it close with the recommendation that you go out and buy the book. It's a cinch you'll have at least to pretend you've read it the next time you dine out.

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